Liquid-Chromatographic Methods for Carboxylic Acids

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Contributor: Makoto Tsunoda

Carboxyl-bearing low-molecular-weight compounds such as keto acids, fatty acids, and other organic acids are involved in a myriad of metabolic pathways owing to their high polarity and solubility in biological fluids. Various disease areas such as cancer, myeloid leukemia, heart disease, liver disease, and lifestyle diseases (obesity and diabetes) were found to be related to certain metabolic pathways and changes in the concentrations of the compounds involved in those pathways. Therefore, the quantification of such compounds provides useful information pertaining to diagnosis, pathological conditions, and disease mechanisms, spurring the development of numerous analytical methods for this purpose.

Keywords: fluorescence ; mass spectrometry ; fatty acids ; perfluorinated carboxylic acids ; α-keto acids

1. Introduction

Quantification of low-molecular-weight compounds, as exemplified by metabolomics studies, has become increasingly important in the life sciences. Metabolite analysis provides metabolic and biochemical status of particular biological systems and valuable insights into disease development and diagnosis [1][2][3][4][5][6]. There are numerous classes of low-molecular-weight compounds, and they are categorized based on their functional groups, including amine, thiol, and carboxylic groups. Low-molecular-weight carboxylic acids are involved in various metabolic pathways. For example, the tricarboxylic acid (TCA) cycle, which is the principal energy-producing process in cells, involves nine carboxylic acid compounds. Fatty acids are integral components of lipids, and consist of carboxylic acids with long aliphatic chains.

Hence, highly sensitive and selective methods for the determination of biologically important carboxylic acids are required for biological investigations, and, thus far, numerous analytical methods have been developed. For selective determination, solid-phase extraction or solvent extraction pretreatment is commonly performed, followed by separation techniques such as liquid chromatography (LC), gas chromatography (GC), and capillary electrophoresis. The choice of detection method is important for trace amounts of carboxylic acids in biological samples. Ultraviolet absorbance detection is rarely implemented due to the absence of chromophores in carboxylic acids. Fluorescence detection following derivatization and mass spectrometry has the advantage of high sensitivity.

2. Analytical methods for fatty acids in biological samples

Target Compounds	Biological Sample	Sample Treatment	Derivatization Reagent	Separation Mode	Detection Method	LOD	Recovery	Ref.
7 Fatty acids	Human serum	Acid extraction	APF	RPLC	FL: 467/512 nm	0.1–6.4 nM	93–105%	[7]
3 Fatty acids	Human plasma	Acid extraction	NOEPES	RPLC	FL: 235/366 nm	56 fmol	–	[8]
6 Fatty acids	Human plasma	Acid extraction	HEC	RPLC	FL: 293/365 nm	38–57 fmol	102–106%	[9]
6 Fatty acids	Human plasma	Acid extraction	HEC	RPLC	FL: 335/365 nm	45–68 fmol	102–105%	[10]
5 Fatty acids	Human serum	Acid extraction	DBD-ED	RPLC	FL: 450/560 nm	2.29–4.75 fmol	108–113%	[11]
8 Fatty acids	Rat plasma	Acid extraction	DBD-ED	RPLC	FL: 450/560 nm	–	–	[12]

4 Epoxyeicosatrienoic acids	Bovine endothelial cells	Solid phase extraction	NT	RPLC	FL: 259/395 nm	<2 pg	83–89%	[13]
25 Fatty acids	Mouse serum	Acid extraction	AMPP	RPLC	MS/MS	50–100 fg (LOQ)	–	[14]
11 Fatty acids	Mouse serum, bronchial epithelial cells	Solid phase extraction	AMPP	RPLC	MS/MS	200–900 fg (LOQ)	–	[15]
20 Fatty acids	Breast cancer cells	Solvent extraction	Aminoxy TMT	RPLC	MS/MS	40 fmol	–	[16]
8 Fatty acids	Rat plasma	Acid extraction	DBD-PZ-NH_2	RPLC	MS	<0.1 μM	–	[17]
9 Fatty acids	Rat plasma	Solvent extraction	DAABD-AE	RPLC	MS	6.5–21 fmol	–	[18]
			MePZBD-AE	RPLC	MS	8.8–32 fmol	–	[18]
			APZBD-NHMe	RPLC	MS	35–150 fmol	–	[18]
56 Fatty acids	Human plasma	Centrifugation	Choline	HILIC	MS	50 ng/mL	–	[19]
38 Fatty acids, acylcarnitines	Human plasma	Centrifugation	Dansyl-hydrazine	RPLC	MS/MS	76–152 pM	–	[20]
18 Fatty acids	Human urine	Solid phase extraction	d_0-DMPP, d_6-DMPP	RPLC	MS/MS	5–15 pM	–	[21]
60 Fatty acids	Human serum	Acid extraction	DMED, d_4-DMED	RPLC	MS	–	–	[22]
6 Fatty acids	Human blood	Acid extraction	None	RPLC	MS	low pg range	–	[23]
4 Fatty acids	Human serum, plasma	Solvent extraction	None	RPLC	ECD	50 pmol	92–102%	[24]
6 Fatty acids	Human plasma	Solvent extraction	None	RPLC	ECD	50 pmol	92–102%	[25]

11 Fatty acids	Human plasma	Solvent extraction	AEMP, NAPP	RPLC	Electrogenerated chemilu minescence	70 fmol	–	[26]

APF: 6-oxy-(acetyl piperazine)fluorescein, NOEPES: 2-(2-naphoxy)ethyl 2-(piperidino)ethanesulfonate, HEC: 9-(2-hydroxyethyl)-carbazole, DBD-ED: 4-*N,N*-dimethylaminosulfonyl-7-*N*-(2-aminoethyl)amino-2,1,3-benzoxadiazole, NT: 2-(2,3-naphthalimino)ethyl trifluoromethanesulfonate, AMPP: *N*-(4-aminomethylphenyl)pyridinium, AminoxyTMT: aminoxy tandem mass tags, DBD-PZ-NH_2: 7-(*N,N*-dimethylaminosulfonyl)-4-(aminoethyl)piperazino-2,1,3-benzoxadiazole, DAABD-AE: 4-[2-(*N,N*-dimethylamino)ethylaminosulfonyl]-7-(2-aminoethylamino)-2,1,3-benzoxadiazole, MePZBD-AE: [4-(4-*N*-methyl)piperazinosulfonyl]-7-(2-aminoethylamino)-2,1,3-benzoxadiazole, APZBD-NHMe: [4-(4-*N*-aminoethyl)piperazinosulfonyl]-7-methylamino-2,1,3-benzoxadiazole, DMPP: 2,4-dimethoxy-6-piperazin-1-yl pyrimidine, DMED: 2-dimethylaminoethylamine, AEMP: 2-(2-aminoethyl)-1-methylpyrrolidine, NAPP: *N*-(3-aminopropyl)pyrrolidine.

3. Analytical methods for TCA cycle and glycolysis-related compounds in biological samples

Target Compounds	Biological Sample	Sample Treatment	Derivatization Reagent	Separation Mode	Detection Method	LOD	Recovery	Ref.
Fumaric acid	Rat liver, spleen and urine	Centrifugation	None	RPLC	PDA: 215 nm	0.01 µg	89–92%	[27]
Maleic acid	Rat serum and urine	Centrifugation	None	RPLC	MS/MS	0.2 µg/L	94–111%	[28]
Methylma-lonic acid	Human plasma	Centrifugation	None	HILIC	MS	0.03 µM	90–93%	[29]
Lactic acid	Human urine and saliva	Centrifugation	9-CMA	RPLC	UV: 365 nm, FL: 365/410 nm	50 nM	92–106%	[30]
Oxalic acid	Mouse urine and hepatocyte	Centrifugation	None	Ion exclu sion chrom atography	MS/MS	2 µM	–	[31]
6 TCA metabolites	Rat urine	Centrifugation	DBD-PZ	RPLC	FL: 450/560 nm	2–15 fmol	80–96%	[32]
9 Organic acids	Yeast	Centrifugation	None	Ion exclu sion chrom atography	UV: 210 nm	0.6–29.3 mg/L	98–103%	[33]
32 Organic acids	Human urine	Solvent extraction	None	Ion exclu sion chrom atography	UV: 220 nm	0.002–2.2 mg/L	–	[34]
13 Organic acids	Mouse urine	Centrifugation	1-Pyrene methylamine	RPLC	FL: 345/375, 345/475 nm	4–22 fmol	–	[35]

30 Organic acids	Mouse serum, urine, and tissue	Centrifugation	None	HILIC, Ion pair RPLC	MS/MS	<5 µM	–	[36]
59 Organic acids	Human melanoma cells	Centrifugation	Phenylh-drazine	Ion pair RPLC	MS	–	–	[37]
138 Organic acids	Yeast	Centrifugation	None	RPLC	MS/MS	0.001–3.7 µM	–	[38]
TCA metabolites	Human red blood cell	Centrifugation	None	RPLC	MS	–	–	[39]

9-CMA: 9-chloromethyl anthracene, DBD-PZ: 7-(N,N-dimethylaminosulfonyl)-4-piperazino-2,1,3-benzoxadiazole.

4. Analytical methods for amino acid metabolites in biological samples

Target Compounds	Biological Sample	Sample Treatment	Derivatization Reagent	Separation Mode	Detection Method	LOD	Recovery	Ref.
Kinurenic acid	Rat plasma	Centrifugation	None	RPLC	FL: 251/398 nm	0.16 nM	97–98%	[40]
3 Trp metabolites	Mouse plasma and brain	Centrifugation	None	RPLC	UV, FL	0.03–1.33 µM	83–116%	[41]
6 Trp metabolites	Pig urine, plasma	Centrifugation	None	RPLC	MS	10–100 ng/mL (LOQ)	–	[42]
Glycated Trp	Chicken plasma	Solvent extraction	None	RPLC	MS	–	–	[43]
PHP-THβC	Chicken plasma	Cation-exchange resin	None	RPLC	MS	–	–	[44]
5 Trp and Tyr metabolites	Human urine	Centrifugation	None	RPLC	UV: 220, 280 nm, FL: 280/350, 315/425 nm	–	–	[45]
DOPAC, HVA	Rat kidney	Microdialysis	Ethylenediamine	Ion exchange chromatography	FL: 417/495 nm	50, 100 fmol	–	[46]

Nicotinic acid	Human plasma	Solvent extraction	None	RPLC	MS/MS	6.57 ng/mL (LOQ)	70–72%	[47]
Glutaric acid, 3-HG	Human urine	Centrifugation	DAABD-AE	RPLC	MS/MS	20–25 nM	94–121%	[48]
64 amino acid derivatives	Human urine, pancreatic cancer cells	Centrifugation	DmPABr	RPLC	MS/MS	0.11–2192 nM	–	[49]

PHP-THβC: (1*R*, 3*S*)-1-(D-gluco-1, 2, 3, 4, 5-pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, DOPAC: 3,4-dihydroxyphenylacetic acid, HVA: homovanillic acid, 3-HG: 3-hydroxyglutaric acid, DAABD-AE: 4-[2-(*N*,*N*-dimethylamino)ethylaminosulfonyl]-7-(2-aminoethylamino)-2,1,3-benzoxadiazole, DmPABr: dimethylaminophenacyl bromide

5. Analytical methods for perfluorinated carboxylic acids (PFCAs) in biological samples

Target Compounds	Biological Sample	Sample Treatment	Derivatization Reagent	Separation Mode	Detection Method	LOD	Recovery	Ref.
3 PFASs	Human tissues and blood	Solid phase extraction	None	RPLC	MS	3 μg/L	80–101%	[50]
10 PFASs	Two bivalves shells, soft tissues	Solid phase extraction	None	RPLC	MS/MS	0.05–0.43 ng/g	92–104%	[51]
18 PFASs	Human urine and serum	Solid phase extraction	None	RPLC	MS/MS	0.1 μg/L	94–104%	[52]
21 PFASs	Human serum	Solid phase extraction	None	RPLC	MS/MS	0.008–0.19 μg/L	85–114%	[53]
6 PFASs	Human plasma	μ-SPE	None	RPLC	MS/MS	21–65 ng/L	88–102%	[54]
6 PFASs	Human serum	Deproteinization	MASH	RPLC	MS/MS	0.07–0.42 μg/L	96–100%	[55]
11 PFASs	Human blood	Solvent extraction	None	RPLC	MS/MS	0.06–0.14 μg/L	67–112%	[56]
20 PFASs	Human plasma, BCS	Centrifugation	None	RPLC	MS/MS	0.024–0.096 μg/L (LOQ)	83–103%	[57]

PFASs: polyfluoroalkyl substances, MASH: 10-methyl-acridone-2-sulfonohydrazide.

6. Analytical methods for α-keto acids and 2-hydroxyglutaric acid (2-HG) in biological samples

Target Compounds	Biological Sample	Sample Treatment	Derivatization Reagent	Separation Mode	Detection Method	LOD	Recovery	Ref.
4 α-Keto acids	Human serum	Centrifugation	OPD	RPLC	FL: 350/410 nm	1 μM	86–109%	[58]
7 α-Keto acids	Human neutrophil	Centrifugation	OPD	RPLC	FL: 360/415 nm	0.035–0.125 μM	79–108%	[59]
3 α-Keto acids	Human CML cell	Gel extraction	OPD	RPLC	FL: 360/415 nm	18–40 nM	84–96%	[60]
6 α-Keto acids	Human CML cell	Centrifugation	DMB	RPLC	FL: 367/446 nm	1.3–5.4 nM	86–118%	[61]
3 α-Keto acids	Mouse tissue	Acid extraction	OPD	RPLC	MS	5 nM	76–95%	[62]
10 α-Keto acids	Rat plasma	Centrifugation	*O*-PFBO	RPLC	MS/MS	0.01–0.25 μM	96–109%	[63]
3 α-Keto acids	Human plasma	Centrifugation	None	RPLC	MS/MS	0.04 μg/mL	81–98%	[64]
(*R*)-2-HG	Human serum	Solid phase extraction	DATAN	RPLC	MS/MS	0.060 μM	31–32%	[65]
(*R*)-2-HG	Human urine, cancer tissues	Solvent extraction	TSPC	RPLC	MS/MS	1.2 fmol	88–109%	[66]

OPD: *o*-phenylenediamine, DMB: 1,2-diamino-4,5-methylenedioxybenzene, *O*-PFBO: *O*-(2,3,4,5,6-pentafluorobenzyl)oxime, DATAN: (+)-*o,o*'-diacetyl-l-tartaric anhydride, TSPC: *N*-(*p*-toluenesulfonyl)-L-phenylalanyl chloride.

7. Analytical methods for 2-aminothiazoline-4-carboxylic acid (ATCA), 2-methylthiazolidine-4-carboxylic acid (MTCA), and 2-thiothiazolidine-4-carboxylic acid (TTCA) in biological samples

Target Compounds	Biological Sample	Sample Treatment	Derivatization Reagent	Separation Mode	Detection Method	LOD	Recovery	Ref.
ATCA	Rat plasma and organ	Solid phase extraction	None	RPLC	MS/MS	–	–	[67]
ATCA	Human urine	MISBSE	None	RPLC	MS/MS	5 μg/L	–	[68]

ATCA	Rat plasma	Solid phase extraction	None	RPLC	MS/MS	12 µg/L	–	[69]
ATCA	Human postmortem blood	Solid phase extraction	None	HILIC	MS/MS	2.5 µg/L	81–89%	[70]
ATCA	Human postmortem blood	Solid phase extraction	None	HILIC	MS/MS	9 µg/L (LOQ)	88–96%	[71]
ATCA	Human post-mortem blood	Liquid-liquid extraction	None	HILIC	MS/MS	0.43 µg/L	86–101%	[72]
MTCA	Human blood and urine	Centrifugation	Acetic anhydride	RPLC	MS/MS	0.1 mg/L	–	[73]
TTCA	Urine	Acid extraction	None	RPLC	UV: 271 nm	35 µg/L	78–87%	[74]

MISBSE: molecularly imprinted stir bar sorption extraction.

8. Analytical methods for other carboxylic acids in biological samples

Target Compounds	Biological Sample	Sample Treatment	Derivatization Reagent	Separation Mode	Detection Method	LOD	Recovery	Ref
7 Bile acids	Human saliva	SPE and solvent extraction	2-Picolylamine	RPLC	MS/MS	1.5–5.6 fmol	–	
3 Bile acids, 8 fatty acids	Human plasma and saliva	Solid phase extraction	APBQ	RPLC	MS/MS	0.19–0.51 fmol	–	
7 Bile acids, 9 fatty acids	Human serum	Solvent extraction	DBCETS	RPLC	FL: 300/395 nm	0.28–0.70 ng/mL	92–102	
4 Bile acids	*C. bovis*	Centrifugation	2-bromo-4′-nitroacetophenone	RPLC	UV: 263 nm	0.25–0.31 ng	94–99%	
7 Bile acids	Human feces	Solid phase extraction	Phenacyl bromide	RPLC	UV: 254 nm	1.22–1.46 pmol	72–102	
	Human feces	Solid phase extraction	None	PRLC	MS/MS	–	–	
Dihydroxyoxocholestenoic acids	Human CSF and plasma	Solid phase extraction	Isotope-label ed Girard's P Reagent	RPLC	MS	0.02–0.05 ng/mL	–	
7 THGC glucuronides	Human urine	Centrifugation	Isotope-labeled DAPPZ	RPLC	MS/MS	0.008–0.16 µg/mL (LOQ)	–	

Orotic acid	Urine	Dilution	None	RPLC	MS/MS	0.15 µM	–
Metabolome	Human urine	Centrifugation	Isotope-label ed DmPABr	RPLC	MS	–	–
Metabolome	Human urine	Centrifugation	Isotope-labeled dansyl hydrazine	RPLC	MS	–	–

APBQ: 1-(3-aminopropyl)-3-bromoquinolinium bromide, DBCETS: 2-(7H-dibenzo[a,g]carbazol-7-yl)ethyl 4-methylbenzenesulfonate, DAPPZ: 1-[(4-dimethylaminophenyl)-carbonyl]piperazine, DmPABr: dimethylaminophenacyl bromide.

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