Journal of Chromatography A, 1446 (2016) 78–90

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Benzoyl chloride derivatization with liquid chromatography–mass spectrometry for targeted metabolomics of neurochemicals in biological samples

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ARTICLE INFO

Article history:
Received 11 February 2016
Received in revised form 28 March 2016
Accepted 2 April 2016
Available online 4 April 2016

Keywords:
Derivatization
HPLC
Mass spectrometry
Neurochemistry
Drosophila
Brain

ABSTRACT

Widely targeted metabolomic assays are useful because they provide quantitative data on large groups of related compounds. We report a high performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) method that utilizes benzoyl chloride labeling for 70 neurologically relevant compounds, including catecholamines, indoleamines, amino acids, polyamines, trace amines, antioxidants, energy compounds, and their metabolites. The method includes neurotransmitters and metabolites found in both vertebrates and insects. This method was applied to analyze microdialysate from rats, human cerebrospinal fluid, human serum, fly tissue homogenate, and fly hemolymph, demonstrating its broad versatility for multiple physiological contexts and model systems. Limits of detection for most assayed compounds were below 10 nM, relative standard deviations were below 10%, and carryover was less than 5% for 70 compounds separated in 20 min, with a total analysis time of 33 min. This broadly applicable method provides robust monitoring of multiple analytes, utilizes small sample sizes, and can be applied to diverse matrices. The assay will be of value for evaluating normal physiological changes in metabolism in neurochemical systems. The results demonstrate the utility of benzoyl chloride labeling with HPLC–MS/MS for widely targeted metabolomics assays.

1. Introduction

Metabolomics is a valuable approach for studying physiological mechanisms and identifying biomarkers. Both untargeted and targeted assays, also called metabolite profiling, are used in such studies. Targeted assays measuring a limited number of metabolites allow focus on important known compounds or pathways and offer better quantification, but they provide lower metabolome coverage compared to untargeted methods. Targeted assays that measure relatively large numbers of compounds (i.e., over 50) help mitigate the disadvantage of limited metabolome coverage. Gas chromatography-mass spectrometry (MS) and high performance liquid chromatography (HPLC)-MS are well-suited platforms for developing such widely targeted assays. Several methods for measuring over 100 known metabolites in a single assay using these techniques have been reported [1–6]. These widely targeted assays are powerful, but they rarely use more than a few internal standards, and for HPLC often make use of ion-pairing reagents [3,6] or multiple LC pumps [4,5] to account for the wide polarity range of the metabolites. Here we report a targeted method for 70 neurochemicals that uses HPLC–MS/MS with benzoyl chloride (BzCl) as a derivatizing agent and avoids these limitations.

HPLC- tandem mass spectrometry (MS/MS) using a triple quadrupole mass spectrometer is well established as a sensitive, quantitative, and selective technique for metabolite profiling [7,8]. Although compounds can be detected by MS/MS without labeling, the use of BzCl provides numerous advantages with only minor drawbacks. In particular, addition of a phenyl group to the polar analytes increases retention on reversed phase columns, which aids resolution and decreases ion suppression. Many compounds are detected with greater sensitivity after labeling, e.g. 1000-fold

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http://dx.doi.org/10.1016/j.chroma.2016.04.006
0021-9673/

Compound Abbreviations

DOMA	3,4-Dihydroxymandelic acid
DOPAC	3,4-Dihydroxyphenylacetic acid
LDOPA	3,4-Dihydroxyphenylalanine
DOPEG	3,4-Dihydroxyphenylglycol
3HAA	3-Hydroxyanthranilic acid
3HK	3-Hydroxykynurenine
MOPEG	3-Methoxy-4-hydroxyphenylglycol
3MT	3-Methoxytyramine
GABA	4-Aminobutyric acid
5HIAA	5-Hydroxyindoleacetic acid
5HTP	5-Hydroxytryptophan
5HTOL	5-Hydroxytryptophol
ACh	Acetylcholine
Ado	Adenosine
Agm	Agmatine
Ala	Alanine
Ans	Anserine
Arg	Arginine
Asn	Asparagine
Asp	Aspartate
BAla	β-Alanine
Carn	Carnosine
Ch	Choline
Cit	Citrulline
CA	Cysteic acid
Cys	Cysteine
DA	Dopamine
E	Epinephrine
ETA	Ethanolamine
Glc	Glucose
Glu	Glutamate
Gln	Glutamine
GSH	Glutathione
Gly	Glycine
Hist	Histamine
His	Histidine
HCA	Homocysteic acid
HCY	Homocysteine
HSer	Homoserine
HVA	Homovanillic acid
HTau	Hypotaurine
KA	Kynurenic acid
Kyn	Kynurenine
Kyo	Kyotorphin
Leu	Leucine
Lys	Lysine
Met	Methionine
NAP	*N*-Acetylputrescine
NAS	*N*-Acetylserotonin
NE	Norepinephrine
NM	Normetanephrine
OA	Octopamine
Orn	Ornithine
Phe	Phenylalanine
PhEt	Phenylethylamine
Pro	Proline
Put	Putrescine
Ser	Serine
5HT	Serotonin
Spd	Spermidine
Spm	Spermine
Syn	Synephrine
Tau	Taurine
Thr	Threonine
TrpA	Tryptamine
Trp	Tryptophan
TyrA	Tyramine
Tyr	Tyrosine
Val	Valine
VMA	Vanillylmandelic acid

increases in sensitivity have been reported for BzCl labeling [9]. The labeling step allows rapid creation of stable-isotope labeled internal standards by using ^{13}C-BzCl for labeling standards, thereby improving quantification for every analyte. BzCl is widely applicable because it derivatizes primary and secondary amines, phenols, thiols, and some alcohols (e.g., ribose hydroxyls and glucose). Indeed, it has previously been used with MS or ultraviolet absorption detection for monitoring neurochemicals in dialysate [9,10], plasma [10], and human cerebrospinal fluid (CSF) [11]. It has also been used for other amine [12,13] and alcohol [14,15] containing compounds. These previous assays targeted a relatively narrow group of compounds.

Although we focus on BzCl, other reagents such as dansyl chloride may provide similar utility for metabolomics [16–19]. We favor BzCl because it reacts faster (seconds at room temperature compared to 20 min at elevated temperature), has a wider pH range for reaction, is less prone to photodegradation, and is commercially available in ^{13}C-labeled form. Additionally benzoylated products are stable for a week at room temperature [9], and standards and internal standards are stable for six months at −80 °C (data not shown).

The 70 compound assay described here targets neurochemicals. Neurons specialize in storing and transmitting information using neurotransmitters and neuromodulators. Low molecular weight polar compounds represent an important class of neurotransmitters including acetylcholine, adenosine, catecholamines, indoleamines, amino acids, trace amines, and dipeptides. A variety of other compounds, such as energy metabolites, antioxidants, and polyamines that affect neuronal function or have been linked to neurological disease are also included in the assay. (A complete list of the compounds included in the assay and their functions is in Supplemental A.) This assay focuses on these compounds and their precursors and degradation products, as their measurement can provide insights into neuronal function to better understand the neurochemical changes in brain diseases. Although this is not a comprehensive assay for all neurochemicals, it demonstrates the wide applicability of BzCl derivatization. The method is an improvement over previous neurochemical assays which were limited to even smaller subsets of neurochemicals [10,20–26], including our previously described 17 compound method based on similar technology [9].

This report demonstrates the utility of BzCl with HPLC–MS for targeted metabolomics on several sample types including tissue, serum, CSF, and microdialysate. Tissue samples are used to characterize concentrations at fixed time points and are best used for determining the overall production and metabolism of neurochemicals. We demonstrate the assay for *Drosophila melanogaster* tissue and hemolymph, an important neurochemical model system. Serum and CSF assays are useful for biomarker studies and assessment of overall physiological state. Microdialysis samples the brain extracellular space and enables the measurement of released neurochemicals over time, making it valuable for correlating neurochemical dynamics to behavior, monitoring drug effects, and