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Determination of amines and phenolic acids in wine with benzoyl chloride derivatization and liquid chromatography–mass spectrometry

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ABSTRACT

Amine and phenolic metabolites are important contributors to the flavor and health effects of many foods, including wine. Determination of these metabolites often involves UV detection following separation by liquid chromatography. While this is sufficient for some applications, chemical derivatization with LC–MS provides greater sensitivity and selectivity relative to LC-UV.

We have developed an assay for 56 amine and phenolic metabolites in wine using benzoyl chloride derivatization and LC–MS. Isotopically labeled benzoyl chloride was used to prepare internal standards for each metabolite. Nanomolar limits of detection were achieved for all metabolites. To demonstrate the application of this assay, we compared metabolite profiles from Merlot and Cabernet Sauvignon wines from California and Australia. We found five metabolites which were significantly different when grouped by varietal, while twenty-four were different when grouped by location of production. This shows that the method can identify differences between various wines.

1. Introduction

Consumers, regulators, and producers are increasingly interested in obtaining information on the characteristics and the quality of food products [1]. This interest has spawned development of a wide variety of methods for analyzing consumable goods (e.g., wine, honey, tea, olive oil and juices) [2]. With respect to wine, various national organizations require strict control over factors such as geographical origin and grape varieties to maintain consistency and quality [3]. Thus, characterization methods are required to assess authenticity and detect wine fraud. Separation techniques such as LC and GC have been widely used for wine characterization and classification. Two important families of LC-amenable wine components are phenols and biogenic amines. Compositional profiles of phenolic and/or amino species have been correlated with significant factors such as organoleptic properties, wine-making practices, and grape varieties [4,5]. In this work, we describe a new approach to assay of phenols and amines in wine using derivatization followed by LC–MS/MS for separation and quantification.

Phenols are a family of bioactive compounds found in wine that have drawn significant attention over the last few years. These aromatic secondary metabolites are ubiquitous in the plant kingdom. They comprise a complex family of more than 8000 substances with highly diverse structures and sizes from <100 Da to >30,000 Da for highly polymerized polyphenolic species. The main reasons for the interest in phenols are their antioxidant properties, great abundance in our diet, probable role in the prevention of various diseases, and contribution to sensorial properties [6–8]. Wine is an excellent natural source of various phenols that range from phenolic acids like benzoic- or cinnamic-like derivatives to different classes of polyphenolic flavonoids such as flavones, flavan-3-ols,

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Compound Abbreviations

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ACh	Acetylcholine
Ado	Adenosine
Agm	Agmatine
Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
βAla	β-Alanine
Cad	Cadaverine
Caf	Caffeic acid
Ch	Choline
Cit	Citrulline
Cou	p-Coumaric aicd
Cys	Cysteine
DA	Dopamine
DOMA	3,4-Dihydroxymandelic acid
DOPA	3,4-Dihydroxyphenylalanine
DOPAC	3,4-Dihydroxyphenylacetic acid
DOPEG	3,4-Dihydroxyphenylglycol
ETA	Ethanolamine
Fer	Ferulic acid
GABA	γ-Aminobutyric acid
Gal	Gallic acid
Glc	Glucose
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
His	Histidine
Hist	Histamine
HVA	Homovanillic acid
Lys	Lysine
Met	Methionine
MOPEG	3-Methoxy-4-hydroxyphenylglycol
NAP	N-Acetylputrescine
Orn	Ornithine
PCA	Protocatechuic acid
Phe	Phenylalanine
PhEt	Phenethylamine
Pro	Proline
Put	Putrescine
Ser	Serine
Sin	Sinapic acid
Spd	Spermidine
Spm	Spermine
Tau	Taurine
Thr	Threonine
TOH	Tyrosol
Trp	Tryptophan
TrpA	Tryptamine
Tyr	Tyrosine
TyrA	Tyramine
VA	Vanillic acid
Val	Valine
VMA	Vanillylmandelic acid
VN	Vanillin
Xle	Leucine/Isoleucine

flavonols and anthocyanins [9]. For this reason, analytical methods such as comprehensive LC techniques have been exploited over the last few years especially to quantify phenols in wine [10–12].

In addition to phenols, biogenic amines have also been the subject of some studies [13–18]. Some of the biogenic amines usually found in wines are agmatine, spermine, spermidine, putrescine, cadaverine, histamine, and tyramine. These compounds are all produced by microorganisms during fermentation via decarboxylation of free amino acids. The consumption of some of them, e.g., histamine and tyramine, can lead to headaches, nausea, hot flushes, skin rashes, sweating, respiratory distress, and cardiac/intestinal problems [19]. Because these components may be responsible for the biological responses to wine consumption, their measurement in different wine varieties of various origins has great importance.

LC with UV detection has been widely used for the determination of amines and phenolic compounds in wine and other beverages. Phenols can be detected in their native state [11,20–22], while amines require derivatization to be compatible with UV detection [23–26]. Although these methods are adequate for measuring a few metabolites, the limited selectivity makes it difficult to characterize a large (e.g., 20+) panel of metabolites, especially in complex mixtures. Mass spectrometry (MS) detection offers a way to overcome these limitations. MS has much better selectivity than UV detection, making it possible to distinguish many more metabolites, even co-eluting compounds, in complex mixtures. Using tandem mass spectrometry (MS/MS) allows for greater confidence in peak identification from unique fragmentation patterns. Additionally, MS/MS is more sensitive than UV, allowing for the measurement of trace metabolites which may not be detected with UV. Mass spectrometry does suffer from instrument drift and matrix effects, but this problem can be corrected through the use of internal standards labeled with stable isotopes.

Some work has been done for the analysis of native amines and phenolic compounds in wine with LC–MS [12,27–29]. However, there are still challenges which much be addressed. Polar amines are poorly retained with reversed phase chromatography, and sensitivity for some trace metabolites may still be limiting. Some of the same derivatization techniques used in UV detection of amines can be beneficial to mass spectrometry and help overcome these challenges. Tagging metabolites with a hydrophobic moiety increases retention of polar metabolites, while also increasing ESI ionization efficiency up to 10,000-fold [30,31]. Additionally, derivatization makes it easy to generate internal standards for each targeted metabolite through the use of stable isotope labeled derivatizing reagents. Labeling improves quantification by accounting for instrument drift and matrix effects, and can aid in peak selection in the presence of background peaks and retention time drift.

Several reagents have been reported that have use for amines and phenols by LC–MS. Derivatization with 1,2-naphthoquinone-4-sulfonate has been used with LC–MS for wine analysis previously [17]. Dansyl chloride derivatization has been used in wine for LC-UV analysis [24]. This same reagent has recently been promoted for the determination of phenols and amine metabolites with LC–MS in a variety of samples including urine, cerebrospinal fluid, and plasma [31–34]. Benzoyl chloride (BzCl) has also been used for LC-UV [35–38] as well as LC–MS [30,39]. Like dansyl chloride, this reagent reacts with amines, phenols, and some hydroxyls. BzCl may have advantages, though, over other reagents for food analysis. The reaction is near instantaneous at room temperature and produces photostable derivatives [30,39]. Furthermore, ^{13}C labeled reagent is readily available at a reasonable cost enabling routine creation of internal standards for all analytes. Here, we demonstrate the application of BzCl derivatization with LC–MS/MS for determination of 56 amine and phenol metabolites in wine. To our knowledge, this method is unique in its capability to measure both amines and phenols in wine in a single, quick assay. Furthermore, the method assays a much larger panel of compounds than other methods,

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