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# PITFALLS DURING QUANTITATIVE DETERMINATION OF METHOXYPYRAZINES IN GRAPE MUST USING GC-MS WITH STABLE ISOTOPE DILUTION ANALYSIS. GCxGC-MS AND ON-LINE LC-(MD)GC-MS AS POTENTIAL LOOPHOLES

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2-Methoxy-3-alkylpyrazines (MPs) are very potent and important flavor compounds found in many fruits and vegetables. They contribute to the green notes in wines made from several *Vitis vinifera* varieties, such as Sauvignon blanc or Cabernet Sauvignon [1]. Whereas 2-methoxy-3-*iso*-butylpyrazine (IBMP) is usually the most abundant MP in must or wine, 2-methoxy-3-*iso*-propylpyrazine (IPMP) is the primary MP from a bug called the Asian ladybeetle (*Harmonia axyridis*) [2]. Quantitative data on presence of these important flavor compounds is of strong concern within the wine industry, as flavor thresholds (and occurrence) are at low ppt level and only a narrow concentration range of MPs is accepted for positive perception in the wine product. In this respect, MP contribution from the Asian ladybeetle, which can be found in great numbers in some regions and under changing climate conditions, has become an important aspect for the wine quality [3] and therefore, analysts seek for reliable analytical methods for MP determination.

In the field of trace level quantification, incorporation of labeled internal standards in a stable isotope dilution analysis (SIDA) approach has often been shown to be beneficial for such analyses. In fact, one of the early SIDA methods described for wine aroma analysis was the quantification of MPs via methoxy-d3-IBMP [4]. During our own approach to the analysis of MPs, we discovered several pitfalls starting from inaccurate description of MP synthesis in literature to critical co-elution problems found with GC-(SIM)-MS after headspace solid phase microextraction (HS-SPME), a method frequently described as sample preparation step for analysis of these compounds [5-8]. Using HS-SPME and comprehensive two-dimensional GC coupled to mass spectrometry (GCxGC-MS) we encountered difficulties in finding undisturbed mass fragments for reliable selected ion monitoring (SIM) analysis. The proposed solution to the co-elution problems found in our matrices is a method based on solid phase extraction, followed by an improved matrix fractionation *via* liquid chromatography (HPLC) as sample pre-separation, coupled on-line to MDGC-(SIM)-MS for final quantitative analysis using labeled MPs as internal standards for quantification.

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