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**Review on sample preparation methods for oligonucleotides analysis by liquid chromatography**

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**Abstract**

Antisense oligonucleotides have been successfully investigated for the treatment of different types of diseases. requires detection and determination of antisense oligonucleotides and their metabolites are necessary for drug development and evaluation. This review focuses mainly on the first step of the analysis of oligonucleotides i.e. the sample preparation stage, and in particular on the techniques used for liquid chromatography and liquid chromatography coupled with mass spectrometry. Exceptional sample preparation techniques are required as antisense oligonucleotides need to be determined in complex biological matrices. The text discusses general issues in oligonucleotide sample preparation and approaches to their solution. The most popular techniques i.e. protein precipitation, protein enzyme digestion and liquid-liquid extraction are reviewed. Solid phase extraction methods are discussed and the issues connected with the application of each method are highlighted. Other newly reported promising techniques are also described. Finally, there is a summary of actually used techniques and the indication of the direction of future research.

*Keywords:* antisense oligonucleotides, liquid chromatography, sample preparation, protein precipitation, enzyme digestion, liquid-liquid extraction, solid phase extraction

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