First Investigations of Quantitative Pencil-Assisted Laser Desorption/Ionisation (PALDI)

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Overview

Pencil lead is an inexpensive, quick and easy to apply, safe MALDI matrix. Here the limits of detection and quantitation of pencil (2B) matrix are compared to classical organic matrices for routine use in a high throughput environment.

Introduction

- Pencil lead has already been shown to be a simple matrix for a number of MALDI applications [1-4]
- 2B has been shown to be the pencil of choice [2]
- It has proved to be a quick and easy method affording qualitative data and removes issues with solvent compatibility of matrix and sample
- This study compares the sensitivity and quantitation of PALDI using 2B pencil matrix with the common organic matrices α–cyanohydroxycinammic acid (α–CHCA) and 2,5-dihydroxybenzoic acid (DHB)

Methods

- DHB (3mg mL^{-1} 50:50 acetonitrile:water 0.1% HCOOH) and α–CHCA as a saturated solution (50:50 acetonitrile:water 0.1% HCOOH) or saturated acetone solution (0.1% HCOOH)
- 1μL of matrix solution pipetted, or pencil scribbled onto the MALDI plate. 1μL of the sample solution pipetted on top of the solid matrix or mixed with the organic/aqueous matrix and left to dry in air
- Data were recorded using Dynamo (Thermo) linear and TofSpec2E (Waters) reflectron MALDI-TOF systems with 337nm nitrogen lasers. 30 laser shots were summed from different positions on the spot by randomly rastering the MALDI plate
- Commercial samples at 1mg mL^{-1} (Substance P, α–Cyclodextrin, Terfenadine) were used without further purification and serially diluted to produce the standard solutions
- Limit of Detection LOD (defined as signal-to-noise >4:1) of the pencil and organic matrices were determined
- Limit of Quantitation LOQ (defined as signal-to noise >5.1) was investigated for Substance P using α–Cyclodextrin as an internal standard

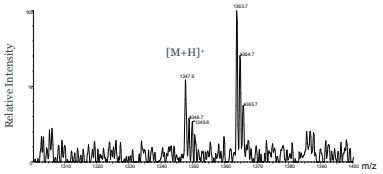


Figure 1: Substance P (0.7 pM uL^{-1}) α– CHCA matrix (Reflectron)

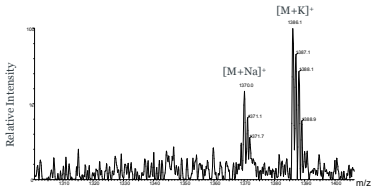


Figure 2: Substance P (7 pM uL^{-1}) 2B matrix (Reflectron)

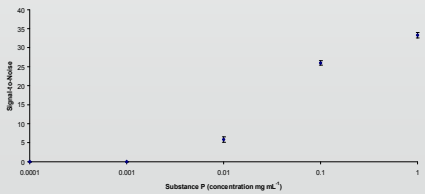
Results and Discussion

- The LOD for Substance P was 0.7 pM μL^{-1} for α–CHCA (acetone 0.1% HCOOH) and 7 pM uL^{-1} for both DHB (0.1% HCOOH) and 2B pencil (no acid) (Figures 1 and 2)
- The LOD for α-Cyclodextrin and Terfenadine using 2B pencil is 10 and 20 pM μL^{-1} respectively and improves by two orders of magnitude using DHB and α–CHCA
- The protonated molecule $[M+H]^+$ is dominant when using the organic matrices and the potassiated molecule $[M+K]^+$ for the pencil matrix
- All matrices give linear responses over at least 3 orders of magnitude sample concentration
- Best analytical practice states that LOQ should only be determined by normalizing to an internal standard. Figure 3 shows the signal-to-noise ratio for Substance P with α–Cyclodextrin present at a concentration of 1mg mL^{-1}

Table 1: LOD for three standards using three different matrices

	Limit of Detection pM μL^{-1}		
Matrix	Substance P (RMM1346 .6)	α–Cyclodextrin (RMM 972.3)	Terfenadine (RMM 471.2)
α-CHCA	0.7	0.1	0.2
DHB	7	0.1	0.2
2B Pencil	7	10	20

Figure 3: Substance P in the presence of α-Cyclodextrin as an internal standard



Conclusions

- Under standard operating conditions the LOD for Substance P using 2B is equivalent to DHB (0.1% HCOOH), showing the application for routine analysis in a high throughput environment (Table 1)
- Ion suppression and preferential ionisation effects due to the presence of an internal standard (Figure 3) causes a significant decrease in signal-to-noise between 0.1 (70 pM uL^{-1}) and 0.01 mg mL^{-1} (7 pM uL^{-1}). This makes confident LOQ difficult to determine using best analytical practice
- These studies have shown that the LOD using 2B pencil matrix can be comparable to standard organic matrices in some cases. For quick analyses, the increased sensitivity gained by a specific classical organic matrix is offset by interference from matrix ions, matrix preparation time and solvent incompatibility issues
- Future work will investigate optimising the concentration of the internal standard and matching internal standard type to the compound of interest to obtain calibration curves for the LOQ using 2B pencil matrix

References

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