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A binary matrix for the rapid detection and characterization of small-molecule cardiovascular drugs by MALDI-MS and MS/MS†

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Abstract

A mixture of α -cyano-4-hydroxycinnamic acid and 1,5-diaminonaphthalene was discovered as a novel binary matrix for the qualitative analysis of 14 small-molecule (~250–550 Da) cardiovascular drugs by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and MS/MS in either positive or negative ion mode.

Since the development of MALDI in the 1980s as a soft ionization technique for biomolecule analysis,^{1–4} it has also been evaluated as a tool for broader applications due to its advantages over electrospray ionization (ESI). MALDI is a high-throughput, sensitive technique that has minimal sample and solvent consumption, requires little instrument configuration, is not sensitive to cross-contamination between samples and provides high-throughput data acquisition rates.^{5–10} Despite these advantages as well as improved instrumentation, MALDI-MS analysis of small molecules (< 500 Da) is still challenging largely due to the interference of chemical background noise in the low m/z region from ions associated with conventional small, organic matrices such as α -cyano-4-hydroxycinnamic acid (CHCA) or 2,5-dihydroxybenzoic acid (DHB) used to help ionize analytes when subjected to laser desorption.^{5,7–10} Therefore, selection of an appropriate matrix (with or without an additive) is crucial to develop a successful MALDI-MS method and is typically determined using a trial and error approach with possible matrices which can be time consuming.

In recent years, efforts have been made to improve the detection of small molecules by MALDI-MS. Most of the advancement has been in lipid analysis and imaging applications,^{6,11–18} while there has been less progress made in the analysis of pharmaceuticals. Using matrix-free approaches or alternative matrices have been examined as methods to detect drugs (*e.g.*, selective instrumental parameters, nanomaterials as matrix alternatives and matrices with molecular weights near 1000 Da). In one study, 53 pharmaceuticals were characterized using a triple quadrupole (QQQ) mass spectrometer with a MALDI source by using selective reaction monitoring (SRM) as an effort to avoid the abundant background

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Conflicts of interest

There are no conflicts to declare.

ions generated by CHCA.¹⁹ However, QQQ mass spectrometers are unit resolution mass spectrometers, therefore, matrix ions can still interfere. In addition, SRM requires known m/z values, which is a limitation when looking for unknown compounds including impurities or contaminants. In a few other small-molecule drug studies, nanomaterials have been evaluated and showed promise as matrix-free alternatives.^{20–23} However, these materials required specialized fabrication which could affect reproducibility and be a bottleneck, inhibiting rapid analysis.

Using an alternative matrix that has a molecular weight greater than those of traditional matrices like CHCA and DHB is an ideal and favourable approach for pharmaceutical analysis by MALDI-MS. *Meso*-tetrakis(pentafluorophenyl)porphyrin (F20TPP) is an example of a metal-binding ligand that has a molecular weight of 975 Da. F20TPP has been used as a matrix to analyze fatty acids, sugars, anabolic steroids and pharmaceuticals.^{10,24–29} When used as a MALDI matrix, only a few matrix peaks due to its fragment ions were observed in the low m/z region.²⁴ In 2006, F20TPP was complexed with Li^+ and successfully ionized 22 out of 26 pharmaceuticals, resulting in low detection limits of 4 pmol or less.¹⁰ The drugs were detected as lithium adducts, $[\text{M} + \text{Li}]^+$, instead of protonated ions, $[\text{M} + \text{H}]^+$.

Although this approach seemed promising, there was no discussion of Li^+ clusters (dimers, trimers, *etc.*), which if present, could reduce sensitivity. Furthermore, MS/MS analysis of Li^+ adducts could affect the fragmentation of the drugs, prohibiting comparison to MS/MS spectral libraries generated from protonated precursor ions. Fragment ions are crucial for structural elucidation and identification, and the effect of Li^+ has not been studied. In addition, the four drugs that went undetected using the Li^+ –porphyrin complex matrix were acetaminophen, trimethoprim, penicillin G and rifampicin. It was unclear why these particular drugs were not detected. Lastly, F20TPP is not applicable with mass spectrometers with relatively long trapping lifetimes such as a Fourier transform ion cyclotron resonance instrument. Metastable fragmentation occurs during trapping time causing interfering signals in the low mass region.³⁰

Another promising matrix alternative is to use a binary matrix mixture consisting of two small organic matrices. Additionally, using a binary matrix results in the formation of protonated or deprotonated ions, which is beneficial for MS/MS analysis. In a previous study, ion pairing agents along with a binary matrix mixture consisting of CHCA and DHB, two acidic matrices, were used to detect phospholipids with molecular weights between 500–1000 Da in both ionization modes.¹⁴ While this matrix combination showed improved signal-to-noise for ions in this mass region, the authors noted the matrices showed an abundance of fragment ions below m/z 500, restricting its use for small-molecular cardiovascular drugs. Using a binary mixture consisting of an acidic and a basic matrix has been shown to reduce matrix clusters and fragment ions in both positive and negative ion modes while increasing signal-to-noise of the analyte.³¹ In a recent study, a mixture of 9-aminoacridine (9-AA) and CHCA was used for analyzing compounds with molecular weights less than 500 Da.³¹ Until now, there have been no reports of using a binary matrix to analyze cardiovascular drugs or other pharmaceuticals. There have been a few cases of

analyzing cardiovascular drugs using a single matrix. These drugs have included metoprolol, verapamil and atorvastatin.^{10,19,32}

In this paper, the development of a binary matrix composed of CHCA and DAN (1,5-diaminonaphthalene) is reported as a novel co-matrix for the analysis small-molecule cardiovascular drugs using a MALDI dual time-of-flight (TOF/TOF) mass spectrometer with MS/MS capability. Compared to using DAN or CHCA separately, the binary matrix works in both ion polarities while generating less interfering matrix signals in the low mass region. Here, using this binary matrix, 14 active pharmaceutical ingredients (APIs) that differ in molecular weight, structure and drug class were successfully detected. Both MALDI-MS and MS/MS were used to analyze drug standards prepared in a single solvent. Therefore, this assessment served as proof of principle that MALDI-MS can be used to rapidly characterize small-molecule drugs universally using a co-matrix and a simple sample preparation solvent.

1. Experimental

1.1. Materials

Atorvastatin calcium, simvastatin, pravastatin sodium, valsartan, losartan potassium, amlodipine besylate, felodipine, lisinopril, hydrochlorothiazide, furosemide, fenofibrate and verapamil hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Rosuvastatin calcium and metoprolol succinate were from BOC Science (Shirley, NY, USA) and USP (Rockville, MD, USA), respectively. 1,5-Diaminonaphthalene, α -cyano-4-hydroxycinnamic acid, 9-aminoacridine, dihydroxybenzoic acid, *p*-nitroaniline (PNA), 2-(4'-hydroxybenzeneazo)benzoic acid (HABA) and diammonium hydrogen citrate (DAHC) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Solvents including acetonitrile (ACN), ethyl acetate, methanol (MeOH), water and hydrochloric acid (HCl) were purchased from Fisher (Fair Lawn, NJ, USA).

1.2. Standard and binary matrix preparation

Stock standard solutions were prepared in MeOH at a concentration of 5 mM. Both CHCA and DAN were prepared at a concentration of 1 mg mL⁻¹ in 1 : 1 (v/v) ACN : water. The optimal binary matrix solution consisted of 1 : 3 (v/v) CHCA : DAN. Stock standard solutions and matrix were mixed 1 : 1 (v/v) and 1 μ L of the mixture was spotted on a polished steel MTP 384 MALDI plate and allowed to dry prior to analysis. For direct infusion ESI-MS and MS/MS analyses, the stock standard solutions were diluted to 0.1 mM in MeOH. An additive solution of 100 mg mL⁻¹ DAHC was prepared in water.

1.3. Desalting pravastatin sodium and losartan potassium

Standard solutions of losartan potassium and pravastatin sodium were separately dissolved in water at 1 mg mL⁻¹. For subsequent liquid-liquid extraction, the solutions were acidified with 10% HCl to pH ~ 1.5. Ethyl acetate was used to extract the free acid drug from the water by first adding 1 mL of ethyl acetate to 1 mL of each acidified drug standard, mixing thoroughly, and allowing the layers to separate. The free acid drug partitioned into the ethyl acetate layer on top leaving the salts behind in the aqueous phase. For analysis by MALDI-MS, the ethyl acetate extracts were diluted with MeOH at 1 : 1 (v/v). The addition of

methanol allowed the extracts to be miscible with the binary MALDI matrix mixture, 1 : 3 (v/v) CHCA : DAN. For direct infusion ESI-MS measurements, the extracts were diluted 5-fold using MeOH.

1.4. Mass spectrometry

MALDI-MS and MS/MS spectra were collected using an ultrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with 2 kHz smartbeam II laser ($\lambda = 355$ nm) and operated in reflectron mode. The method employed to analyze the drug standards used an m/z range of 100–980 in both positive and negative ion modes. MS parameters consisted of the following: ion source 1 voltage: 19.95 kV; ion source 2 voltage: 17.85 kV; lens voltage: 7.04 kV; pulsed ion extraction time: 150 ns; reflector 1 voltage: 21.26 kV and reflector 2 voltage: 10.72 kV. For MS/MS analyses, the ion source, lens and reflector voltages remained the same as MS conditions, but the following instrument parameters changed. The pulse ion extraction time was 90 ns and lift 1 and 2 were 18.97 and 4.49 kV, respectively.

Direct infusion positive and negative ion ESI-MS and MS/MS experiments were conducted using a Thermo Q Exactive mass spectrometer (see ESI† for parameters).

2. Results and discussion

2.1. Development of a binary matrix for low mass region

To develop a rapid and universal MALDI-MS method for commonly used small-molecule cardiovascular drugs on the market, several MALDI matrices, including CHCA, DAN, DHB, 9-AA, PNA, and HABA, were tested. Not surprisingly, when a single matrix was used, an abundance of matrix-related background peaks interfered with signals from the drugs. Another drawback to using matrices individually is that in most cases this limits the instrument to be used in a single polarity, either positive or negative ion mode. Therefore, a single matrix cannot be universal to all drugs since they differ in structure and functional groups, which influence their detection as protonated or deprotonated species. To overcome these shortcomings, a binary matrix containing CHCA and DAN (Fig. 1) was developed after screening different combinations of frequently used matrices, including a binary matrix mixture of CHCA and 9-AA that was reported elsewhere in 2007.³¹ Guo *et al.* demonstrated that this co-matrix showed improved detection for some small molecules and suggested that this was based on their pK_a values relative to those of the matrices. These authors noted that the binary matrix is not universal to all small molecules, but the matrices can be tailored for the analyte of interest. In the current study, preliminary experiments determined that CHCA and 9-AA would not be a suitable co-matrix for the detection of cardiovascular drugs. For example, atorvastatin, a carboxylate-containing drug with a molecular weight of 558 Da, was analyzed using this binary matrix and no analyte signal was observed in the negative-ion MALDI mass spectrum (data not shown). The lack of analyte signal was due to the presence of CHCA in the binary matrix because when atorvastatin was analyzed using only 9-AA, it was detected as a deprotonated ion (m/z 557, $[M - H]^-$). Presumably, because CHCA has a smaller pK_a than that of atorvastatin ($pK_a \sim 4.0$ – 4.5),^{33–35} its signal was suppressed by the matrix. The competing nature of CHCA for ionization in negative-ion mode could limit its

use as an acidic matrix in a binary mixture. Despite the fact that CHCA in combination with 9-AA prevented the detection of atorvastatin, CHCA as an acidic matrix offers several benefits, including better signal-to-noise and fewer background ions as described by Guo *et al.*³¹ Based on the literature, CHCA has been the most frequently used matrix for small-molecule drug analysis in positive-ion mode.^{9,14,36,37} Additionally, preliminary research showed that CHCA is a promising matrix for the detection of cardiovascular drugs in positive-ion mode. Thus, instead of replacing CHCA in the binary mixture for a different acidic matrix, substituting 9-AA with a basic alternate was evaluated.

DAN is a basic matrix and its conjugate acid has lower pK_a values ($pK_{a1} \sim 4.6$, $pK_{a2} \sim 2.6$) than that of 9-AA ($pK_a = 10.2$).^{31,38,39} In two recent studies, DAN was used in lipid and small-molecule metabolite research to detect negatively charged ions.^{17,40–42} In 2012, DAN was used to analyze lipids (negative ion m/z range of 650–1600) and compared the results to 7 other matrices, including 9-AA.⁴¹ Thomas and co-workers found that DAN provided better sensitivity over the other matrices, and suggested DAN's reductive properties promotes deprotonation by being a gas-phase “proton-pump”.^{41,43} Two years later, Korte *et al.* compared DAN to 9-AA for the detection of small-molecule metabolites and found DAN provided better sensitivity for compounds with molecular weights less than 400 Da.¹⁷ These literature findings as well as preliminary experiments using DAN as a single matrix to analyze atorvastatin in the negative-ion mode lead to the current evaluation of using CHCA and DAN as a binary matrix for the qualitative assessment of 14 cardiovascular drugs.

Unlike other MALDI matrices, the major peaks associated with DAN are odd-electron molecular ions.^{42,43} DAN was analyzed using positive and negative ionization as depicted in Fig. 2. In positive-ion mode, DAN favored a radical ion $[M]^{+\bullet}$ at m/z 158, while the ion at m/z 314 was due to $[2M - 2H]^{+\bullet}$ (Fig. 2A).^{42,43} In negative-ion mode, the peak at m/z 157 corresponded to the deprotonated molecular ion $[M - H]^-$ while m/z 312 resulted from a radical ion $[2M - 4H]^{-\bullet}$ (Fig. 2B).^{42,43} Additional ions at m/z 299 $[2M - NH_3]^{+\bullet}$ and m/z 298 $[2M - NH_4]^{-\bullet}$ most likely originated from a reaction between neutral DAN molecules and radical ions.⁴³

Even though CHCA has been the most commonly used matrix for small-molecule drug analysis in positive-ion mode,⁹ several matrix-derived peaks were observed in the low m/z region (Fig. 2C). In the positive-ion mass spectrum, the most intense peaks were a pseudomolecular ion $[M + H]^+$ at m/z 190, a fragment ion at m/z 172 due to the loss of H_2O and a dimer $[2M + H]^+$ at m/z 379. In addition to these ions, there were several less abundant signals present. Three of these peaks included a sodium adduct at m/z 212 $[M + Na]^+$, a fragment ion at m/z 146 due to loss CO_2 and a cluster of $[2M - CO_2 + H]^+$ at m/z 335. There were trimer clusters at m/z 524 $[3M - CO_2 + H]^+$ and 568 $[3M + H]^+$. DAHC was added to the CHCA matrix in order to suppress background matrix peaks.⁴⁴ However, when this additive was used to analyze drugs their signals were also suppressed. Comparatively, the negative-ion mass spectrum of CHCA in Fig. 2D is relatively simple with two predominate peaks at m/z 188 and 779 due to a carboxylate ion $[M - H]^-$ and presumably a cluster ion, respectively. Less intense ions at m/z 144, 289, 333, 590, 620 and 967 were also observed. The ions at m/z 144, 289 and 333 are due to $[M - CO_2 - H]^-$, $[2M$

$-2\text{CO}_2\text{-H}]^-$ and $[2\text{M} - \text{CO}_2\text{-H}]^-$, respectively. Additional cluster ions were observed at m/z 590, 620 and 967.

When CHCA and DAN were co-mixed as a binary matrix and analyzed, favourable ion suppression was observed in both ionization modes presumably as a result of proton transfer from CHCA to DAN. Only one predominate peak was observed in the positive ion mode at m/z 158 (Fig. 2E) while no DAN matrix peaks were observed. In the negative ion mass spectrum, only a single peak due to CHCA at m/z 188 (Fig. 2F) can be seen and no matrix ions from DAN were detected. Therefore, the decrease in the number of matrix peaks simplified the mass spectra. The optimal binary mixture was composed of 0.25 mg mL^{-1} of CHCA and 0.75 mg mL^{-1} of DAN in 1 : 1 (v/v) ACN : water, which was determined by mixing and analyzing different ratios of CHCA and DAN (both 1 mg mL^{-1}) to obtain the greatest signal-to-noise (S/N) of the drug by minimizing the number of matrix ions observed in both the positive and negative ion modes. The ratio was determined by studying three popular cardiovascular drugs that differ in class, molecular weight and structure, atorvastatin (statin, MW = 558 Da), metoprolol (beta blocker, MW = 267 Da) and valsartan (angiotensin II receptor blocker, MW = 435 Da). Once the ratio of CHCA and DAN was determined for these three example drugs, the binary matrix was used to qualitatively assess the detection and characterization of 11 other cardiovascular drugs to show that it can be a universal co-matrix.

2.2. Binary matrix works in dual polarities

The reduced background noise in the low m/z region from the binary matrix afforded improved mass spectra of all 14 small-molecule pharmaceuticals. Out of the 14 drugs analyzed, metoprolol had the lowest molecular weight (267 Da) and its enhanced detection was demonstrated in Fig. 3. Metoprolol was separately analyzed with CHCA (Fig. 3A) and the binary matrix (Fig. 3B) in positive ion mode.

Metoprolol was detected as a protonated ion at m/z 268 when either the binary matrix or CHCA alone was used. However, in Fig. 3A, multiple CHCA matrix peaks at m/z 172, 190, 212 and 379 were abundant. Using a binary matrix resulted in a mass spectrum that contained only two matrix peaks from DAN at m/z 158 and 314 which were completely separated from metoprolol (Fig. 3B). This is a clear case of how the use of the MALDI binary matrix reduced background matrix ions. Additionally, although the peak intensity of metoprolol decreased by roughly half, S/N increased slightly by 11% and resolution of the peak more than doubled when the binary matrix was used due to the reduction in background noise. These two factors are critical for improved detection and identification.

As mentioned previously, a major drawback of using an individual matrix instead of a co-mixture is that matrices are most often preferential to one polarity. For example, DAN works in negative ion while CHCA works in positive ion mode, preventing their individual application as a universal matrix for cardiovascular drug characterization.^{41,45} A co-mixture of a basic and acidic matrix, however, overcomes this limitation. The binary matrix developed here worked in both positive and negative ion modes for 14 cardiovascular drugs that contained a variety of functional groups in their structures (see ESI, Fig. S-1†).

For example, as shown previously, metoprolol dissolved in methanol was detected in positive ion using a combination of CHCA and DAN, and using this same binary matrix, hydrochlorothiazide was detected as a negatively charged ion owing to its relatively acidic sulfonamide structure (Fig. 4A). Hydrochlorothiazide was detected as a deprotonated species $[M - H]^-$ at m/z 296 and the hydrochlorothiazide chlorine isotopic distribution pattern was observed in the mass spectrum (peaks at m/z 296 and 298 have a 3 : 1 abundance ratio due to ^{35}Cl and ^{37}Cl). An interesting note is that if DAN had been used alone instead of as a mixture with CHCA, then a matrix ion (m/z 298 from DAN shown in Fig. 1B) would have interfered in the analysis of the chlorine isotopic distribution. In addition, using a binary matrix, the negative ion MS/MS spectrum of hydrochlorothiazide was collected by isolating and fragmenting the precursor ion $[M - H]^-$ at m/z 296. The fragment ion mass spectrum (Fig. 4B) showed 4 predominate product ions at m/z 294, 269, 232 and 205.

2.3. Universal MALDI-MS method for 14 cardiovascular drugs

For simplicity, both metoprolol and hydrochlorothiazide were prepared in methanol prior to analysis by MALDI-MS. To test the applicability of using this solvent to dissolve additional small-molecule drugs, 12 more APIs of cardiovascular drugs were evaluated. In addition, the same binary MALDI matrix mixture of CHCA and DAN was used to determine if the binary mixture was a suitable universal matrix for cardiovascular drugs. In total, 14 small-molecule drugs from 7 classes were studied using this universal MALDI method and results are listed in Table 1. All 14 drugs were analyzed in dual polarities. Six of the drugs exhibited more abundant signals in negative ion mode while the other 8 were preferentially detected in positive ion mode. Atorvastatin calcium, rosuvastatin calcium, valsartan, felodipine, hydrochlorothiazide and furosemide were detected as deprotonated ions while simvastatin, pravastatin sodium, metoprolol succinate, losartan potassium, amlodipine besylate, verapamil, lisinopril and fenofibrate were either observed as $[M + H]^+$ or $[M + \text{Na}]^+$. Interestingly, amlodipine was partially labile and several fragment ions were observed when it was analyzed by both MALDI-MS and ESI-MS, but the fragments and their abundances differed between the two ionization techniques (mass spectra not provided). In the MALDI mass spectrum, this calcium channel blocker showed 2 predominate peaks. The most abundant peak was an intact sodium adduct at m/z 431, while the other peak at m/z 407 was due to $[M - 2H + H]^+$, a fragment ion with a relative intensity of 94%, formed *via* oxidation to a pyridine moiety.⁴⁶ When amlodipine was analyzed by ESI-MS, the spectrum also showed several fragment ions including a peak at m/z 407, but its relative abundance was only 1% compared to the pseudomolecular ion at m/z 409, $[M + H]^+$ (mass spectra not provided). Thus, the oxidative fragmentation observed in the MALDI mass spectrum most likely occurred when amlodipine was irradiated with the UV laser instead of resulting from in-solution degradation.

In addition to collecting MS data for each drug, MALDI-MS/MS spectra were also acquired and are provided in the ESI in alphabetical order (Fig. S-2[†]). The last column in Table 1 lists fragment ions observed for each drug with the exception of amlodipine because there were a limited number of fragment ions observed for its sodium adduct. Consequently, the peak at m/z 407 ($[M - 2H + H]^+$) was selected as a precursor ion. ESI-MS/MS was also used to analyze all 14 drugs (Fig. S-3[†]) and the fragment ions were compared to those observed in

the MALDI tandem mass spectra. The majority of fragment ions generated by MALDI were also observed by ESI-MS/MS. This demonstrates that MALDI-MS/MS can be used for small-molecule drug characterization and identification.

One interesting phenomenon observed with the statin class of drugs was that losartan potassium and pravastatin sodium analyzed “as is” prepared in methanol were solely detected in positive ion instead of negative ion (Fig. 5A and C). This was surprising because the structures of these two drugs contain either a carboxylate or tetrazolate. A drug with one of these functional groups would typically favor detection in negative ion mode and such was the case with atorvastatin calcium and rosuvastatin calcium. Lack of signals in the negative ion mode suggested that the abundance of monovalent alkali metal ions present in the standard solutions of losartan and pravastatin were deterring their detection in negative ion mode. Specifically, peaks due to alkali adducts were abundant in the mass spectra of both drugs. In the case of pravastatin, only sodium adducts at m/z 447 and 469 were detected without the presence of a pseudomolecular ion $[M + H]^+$ at m/z 425 (Fig. 5A). For losartan potassium, the pseudomolecular ion $[M + H]^+$ peak with an m/z of 423 was significantly less intense compared to the dipotassium adduct peak with an m/z 499 (Fig. 5C). To test the hypothesis that the abundance of sodium and potassium were suppressing negative ion signals of the drugs, both standards were desalted and subsequently analyzed with positive and negative ion MALDI-MS.

Desalting losartan potassium and pravastatin sodium using a liquid–liquid extraction resulted in the negative ion MALDI mass spectra shown in Fig. 5C and D. Both drugs as free acids were detected as deprotonated species in the negative ion mode. Losartan showed a strong signal at m/z 421 while pravastatin gave rise to a peak at m/z 423, which were both due to $[M - H]^-$. Their deprotonated ions were further fragmented and their corresponding MS/MS spectra were provided in Fig. S-4.† This before and after desalting comparison revealed having a universal MALDI matrix that works in both ion polarities for drug characterization is advantageous.

3. Conclusions

MALDI-MS and MS/MS were used to analyze small-molecule cardiovascular drugs prepared in methanol. A mixture of α -cyano-4-hydroxycinnamic acid (CHCA) and 1,5-diaminonaphthalene (DAN) was developed as a binary MALDI matrix for their detection in either positive or negative ion mode. These drugs ranged in molecular weight (200–600 Da), varied in structures, and covered 7 classes of cardiovascular drugs. This assessment served as proof of principle that MALDI-MS can be used to qualitatively characterize small-molecule drugs using a universal co-matrix and sample preparation solvent. Therefore, MALDI mass spectrometry shows promise as a tool for rapidly screening drug products for quality control issues, including discrepancies in APIs, their dosage strengths and impurities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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α-cyano-4-hydroxycinnamic acid
CHCA (MW=189 Da)

1,5-diaminonaphthalene
DAN (MW=158 Da)

Fig. 1.
Structures of CHCA and DAN.

Fig. 2.

Positive (left) and negative (right) ion MALDI mass spectra of (A and B) DAN, (C and D) CHCA and (E and F) binary matrix of 0.25 mg mL^{-1} of CHCA and 0.75 mg mL^{-1} of DAN in 1 : 1 (v/v) ACN : water.

Fig. 3.
Positive ion MALDI mass spectra of metoprolol using (A) CHCA and (B) binary matrix.

Fig. 4. Negative ion MALDI mass spectra of hydrochlorothiazide with a binary matrix using (A) MS and (B) MS/MS (product ions of m/z 296).

Fig. 5. Positive (top) and negative (bottom) ion MALDI-MS spectra using a binary matrix (A) pravastatin sodium (B) pravastatin free acid (C) losartan potassium and (D) losartan free acid.

Summary of results from MALDI-MS and MS/MS of 14 cardiovascular drugs from 7 classes dissolved in methanol and studied using a binary matrix mixture of CHCA and DAN. Note that the precursor ion selected for the fragmentation of amlodipine besylate was $[M - 2H + H]^+$ (m/z 407) instead of $[M + Na]^+$ (m/z 431). Neg = negative ion and Pos = positive ion

Table 1

Class	Drug	Mode	Protonated or deprotonated ion observed	Precursor m/z	Fragment ions
Statins	Atorvastatin calcium	Neg	$[M - H]^-$	557	539, 521, 479, 453, 397
	Simvastatin	Pos	$[M + Na]^+$	441	425, 353, 325
	Pravastatin sodium	Pos	$[M + Na]^+$	447	429, 345, 327, 309
Beta blocker	Rosuvastatin calcium	Neg	$[M - H]^-$	480	462, 418, 402, 392, 376, 322, 244
	Metoprolol succinate	Pos	$[M + H]^+$	268	250, 226, 191, 177, 116
Angiotensin II receptor blocker	Valsartan	Neg	$[M - H]^-$	434	391, 350, 304, 235, 192, 179
	Losartan potassium	Pos	$[M + H]^+$	423	405, 377, 341, 235, 207, 192, 171
Calcium channel blocker	Amlodipine besylate	Pos	$[M + Na]^+$	407	390, 377, 346, 294, 238, 286
				$[M - 2H + H]^+$	
Angiotensin-converting enzyme inhibitor	Felodipine	Neg	$[M - H]^-$	382	350, 336, 236, 145
	Verapamil	Pos	$[M + H]^+$	455	428, 386, 303, 289, 260, 165, 151
	Lisinopril	Pos	$[M + H]^+$	406	389, 360, 343, 309, 291, 263, 246, 116
Diuretic	Hydrochlorothiazide	Neg	$[M - H]^-$	296	294, 269, 232, 205
	Furosemide	Neg	$[M - H]^-$	329	311, 285, 249, 205
Fibrate	Fenofibrate	Pos	$[M + H]^+$	361	319, 273, 233, 139