Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Matrix-assisted laser desorption/ionization tandem mass spectrometry for identification of organic tattoo pigments in inks and tissue samples

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Supplementary Tables

Table S1 Macro for MS spectra processing integrated into the FlexAnalysis program. The macro includes the commands for integration, baseline subtraction and exportation as Mascot Generic Format (*.MGF).

```
'-----------------------------------------------------------------------------------------------
' Name:
'
' Purpose: Export peaklist to MGF

'
' Date: September 2013
'
' Version: 1.0 (rp)
'-----------------------------------------------------------------------------------------------

Option Explicit
Option Base 0

' Usage:
' cCollectFiles defines if the MGF-files shall be stored in one central output directory or in the folder
next to the fid file
' Const cCollectFiles = True stores all MGF files in the directory specified in cOutputDir
' cCollectFiles = False store the MGF files next to FID

Const cCollectFiles = True
Const cOutputDir = "D:\Data\MGFFiles\"

Dim oFSO As Object


Sub Main
'
' Enter your commands here...
UndoAllProcessing()                                    ' Make sure that further processing starts
from the raw data

Dim dRange(1) As Double                                ' Declare a variable for peak finding range
dRange(0) = 100
dRange(1) = 4000                                ' Range for peak finding: smallest mass in spectrum ->
parent mass + 3 Da

Dim oSpectrum As Spectrum                              ' Declare a spectrum object
Set oSpectrum = ResultSpectra(1).Process(faSubtractBaseline)
Set oSpectrum = ResultSpectra(1).Process(faFindPeaks, dRange) ' Find peaks in the defined mass
range

oSpectrum.Enabled = True                               ' Display result spectrum

Save                                                   ' Save the result spectrum

ExportToMGF
```

```
End Sub
Sub ExportToMGF

Dim oTextStream As Object
Dim OutPutDir As String
   Dim FileName As String
   Dim n As Integer

Set oFSO = CreateObject("Scripting.FileSystemObject")

If cCollectFiles = True Then
   OutPutDir = cOutputDir

    If oFSO.FolderExists(OutPutDir) = False Then
        CreateFolder(OutPutDir)
    End If

    FileName = Name
    FileName = Replace$(FileName, "\", "_")
    FileName = OutPutDir + FileName + ".MGF"
Else

    FileName = Name
    FileName = Replace$(FileName, "\", "_")
    FileName = FileName + ".MGF"


      FileName = GetOutPutDir() + FileName

End If

   '
   ' Create Outpufile
   '

Set oTextStream = oFSO.OpenTextFile(FileName, 2, True)       ' Open for Write

   oTextStream.Writeline("Name: Pigment")
   oTextStream.Writeline("Fomula: A")
   oTextStream.Writeline("MW: A")
   oTextStream.Writeline("PRECURSORMZ:A")
   oTextStream.Writeline("CAS#: A")
oTextStream.Writeline("Comments: NIST Mass Spectrometry Data Center")
   oTextStream.Writeline("Num Peaks: 100")


Dim oPeakList As MassList
Set oPeakList = ResultSpectra(1).MassList

Dim i As Integer
Dim LineOut As String

For i = 1 To oPeakList.Count
    Dim oPeak As MassPeakInfo
    Set oPeak = oPeakList(i)
    LineOut = Str(oPeak.MassToChargeValue) + " " + Str(oPeak.Intensity)
```

```
    oTextStream.WriteLine(LineOut)
Next i
'oTextStream.Writeline("END IONS")
    oTextStream.Close


End Sub
Private Function GetOutPutDir()

   Dim sFolder As String
   sFolder = FullName + "\1SRef"
If oFSO.FolderExists(sFolder) Then
    GetOutPutDir = sFolder + "\"
    Exit Function
End If
sFolder = FullName + "\1SLin"
If oFSO.FolderExists(sFolder) Then
    GetOutPutDir = sFolder + "\"
    Exit Function
End If
sFolder = FullName + "\1Ref"
If oFSO.FolderExists(sFolder) Then
    GetOutPutDir = sFolder + "\"
    Exit Function
End If
sFolder = FullName + "\1Lin"
If oFSO.FolderExists(sFolder) Then
    GetOutPutDir = sFolder + "\"
    Exit Function
End If

End Function

Private Sub CreateFolder(sPath As String)
If sPath <> "" Then
    If Not oFSO.FolderExists(oFSO.GetParentFolderName(sPath)) Then
        CreateFolder(oFSO.GetParentFolderName(sPath))
    End If
    oFSO.CreateFolder(sPath)
End If
End Sub
```

Table S2 Macro for MS/MS spectra processing integrated into the FlexAnalysis program. The macro includes the commands for integration, baseline subtraction, smoothing and exportation as Mascot Generic Format (*.MGF).

```
'-----------------------------------------------------------------------------------------------
' Name:
'
' Purpose: Export peaklist to MGF

'
' Date: September 2013
'
' Version: 1.0 (rp)
'-----------------------------------------------------------------------------------------------

Option Explicit
Option Base 0

' Usage:
' cCollectFiles defines if the MGF-files shall be stored in one central output directory or in the folder
next to the fid file
' Const cCollectFiles = True stores all MGF files in the directory specified in cOutputDir
' cCollectFiles = False store the MGF files next to FID

Const cCollectFiles = True
Const cOutputDir = "D:\Data\MGFFiles\"

Dim oFSO As Object


Sub Main
'
' Enter your commands here...
UndoAllProcessing()                                    ' Make sure that further processing starts
from the raw data

Dim dRange(1) As Double                                ' Declare a variable for peak finding range
dRange(0) = 55
dRange(1) = ParentMass + 4                             ' Range for peak finding: smallest mass in
spectrum -> parent mass + 3 Da

Dim oSpectrum As Spectrum                              ' Declare a spectrum object
Set oSpectrum = ResultSpectra(1).Process(faSmooth)
Set oSpectrum = ResultSpectra(1).Process(faSubtractBaseline)
Set oSpectrum = ResultSpectra(1).Process(faFindPeaks, dRange) ' Find peaks in the defined mass
range

oSpectrum.Enabled = True                               ' Display result spectrum

Save                                                   ' Save the result spectrum

ExportToMGF


End Sub
Sub ExportToMGF
```

```
Dim oTextStream As Object
Dim OutPutDir As String
   Dim FileName As String
   Dim n As Integer

Set oFSO = CreateObject("Scripting.FileSystemObject")

If cCollectFiles = True Then
   OutPutDir = cOutputDir

    If oFSO.FolderExists(OutPutDir) = False Then
        CreateFolder(OutPutDir)
    End If

    FileName = Name
    FileName = Replace$(FileName, "\", "_")
    FileName = OutPutDir + FileName + ".MGF"
Else

    FileName = Name
    FileName = Replace$(FileName, "\", "_")
    FileName = FileName + ".MGF"


      FileName = GetOutPutDir() + FileName

End If

   '
   ' Create Outpufile
   '

Set oTextStream = oFSO.OpenTextFile(FileName, 2, True)       ' Open for Write

   oTextStream.Writeline("Name: Pigment")
   oTextStream.Writeline("Fomula: A")
   oTextStream.Writeline("MW: A")
   oTextStream.Writeline("PRECURSORMZ:" + Str$(ParentMass))
   oTextStream.Writeline("CAS#: A")
oTextStream.Writeline("Comments: NIST Mass Spectrometry Data Center")
   oTextStream.Writeline("Num Peaks: 100")


Dim oPeakList As MassList
Set oPeakList = ResultSpectra(1).MassList

Dim i As Integer
Dim LineOut As String

For i = 1 To oPeakList.Count
    Dim oPeak As MassPeakInfo
    Set oPeak = oPeakList(i)
    LineOut = Str(oPeak.MassToChargeValue) + " " + Str(oPeak.Intensity)
    oTextStream.WriteLine(LineOut)
Next i
'oTextStream.Writeline("END IONS")
```

```
    oTextStream.Close


End Sub
Private Function GetOutPutDir()

   Dim sFolder As String
   sFolder = FullName + "\1SRef"
If oFSO.FolderExists(sFolder) Then
    GetOutPutDir = sFolder + "\"
    Exit Function
End If
sFolder = FullName + "\1SLin"
If oFSO.FolderExists(sFolder) Then
    GetOutPutDir = sFolder + "\"
    Exit Function
End If
sFolder = FullName + "\1Ref"
If oFSO.FolderExists(sFolder) Then
    GetOutPutDir = sFolder + "\"
    Exit Function
End If
sFolder = FullName + "\1Lin"
If oFSO.FolderExists(sFolder) Then
    GetOutPutDir = sFolder + "\"
    Exit Function
End If

End Function

Private Sub CreateFolder(sPath As String)
If sPath <> "" Then
    If Not oFSO.FolderExists(oFSO.GetParentFolderName(sPath)) Then
        CreateFolder(oFSO.GetParentFolderName(sPath))
    End If
    oFSO.CreateFolder(sPath)
End If
End Sub
```

Table S3 Scheduled precursor list for MS/MS acquisition. Characteristic fragment ion peaks (marked with "b" or "c") already present in the MS spectra were also used for pigment identification in addition to the molecular ion peak. The corresponding *m/z* values are given below with alphabetic labels. Abbreviations: P.B. = Pigment Blue; P.Br. = Pigment Brown; P.G. = Pigment Green; P.O. = Pigment Orange; P.R. = Pigment Red; P.Y. = Pigment Yellow; P.V. = Pigment Violet.

Pigment	**Color Index**	***m/z***	**Pigment**	**Color Index**	***m/z***
P.O.13c	21110	251.15	P.O.16b	21160	431.15
P.Y.151b	13980	255.07	P.O.13b	21110	437.14
P.Y.74b	11741	264.10	P.V.1	45170:2	443.20
P.R.88b, P.R.179b	73312, 71130	268.22	P.R.177	65300	445.08
P.Y.14c, P.R.202b	21095, 73907	276.20	P.R.22	12315	449.08
P.V.19	73900	313.16	P.Y.14b	21095	453.06
P.R.170b	12475	318.16	P.R.88	73312	456.79
P.R.4	12085	328.06	P.R.210	12477	463.14
P.Y.97b	11767	329.00	P.R.170	12475	477.19
P.R.22b	12315	334.07	P.R.9	12460	488.00
P.Y.3b	11710	335.03	P.R.112	12370	505.95
P.O.5	12075	339.06	P.B.16, P.Br.25	74100, 12510	514.17
P.R.122	73915	341.12	P.B.15	74160	575.18
P.R.9b, P.Br.25b	12460	342.99	P.V.23	51319	588.06
P.R.254	56110	357.08	P.Y.97	11767	613.04
P.Y.1	11680	363.11	P.O.16	21160	620.19
P.O.71, P.R.112b	561200, 12370	377.07	P.O.13	21110	623.17
P.R.254b	56110	379.05	P.R.146	12485	633.08
P.R.202	73907	381.03	P.R.5	12490	649.10
P.Y.74	11741	387.10	P.Y.14	21095	656.10
P.R.181	73360	393.04	P.O.34	21115	673.12
P.O.34b	21115	396.15	P.Y.138	56300	694.88
P.V.1c	45170:2	399.22	P.V.37	51345	727.25
P.O.73	561170	401.22	P.V.37b	51345	749.17
P.Y.151	13980	404.06	P.Y.83b	21108	818.05
P.Y.154	11781	406.08	P.Y.83	21108	839.19
P.O.43	71105	412.06	P.Y.83c	21108	979.34
P.V.1b	45170:2	415.20	P.G.7	74260	1126.55
P.Y.3	11710	417.01	P.G.36	74265	1661.93
P.R.179	71130	418.08	P.G.36b	74265	1705.88
P.Y.154b	11781	428.05			

Supplementary Figures

Fig. S1 Ionization efficiency of P.B.15 and P.R.202 in MALDI-MS analysis using different matrices. A), B) MALDI-MS spectra of P.B.15 and P.R.202 applied as dried-droplet using EtOH (left), CPA (middle) and SA (right). B) For P.R.202, the molecular ion *m/z* 381 was additionally displayed (upper right corner). C), D) Molecule structures of P.B.15 and P.R.202, respectively.

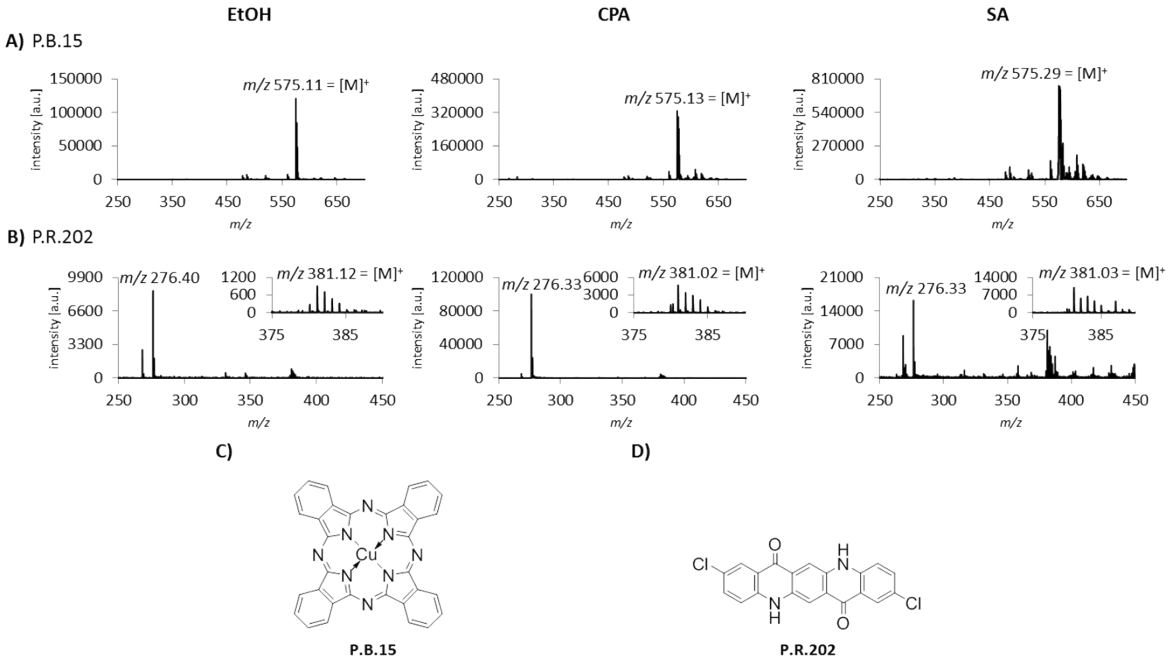
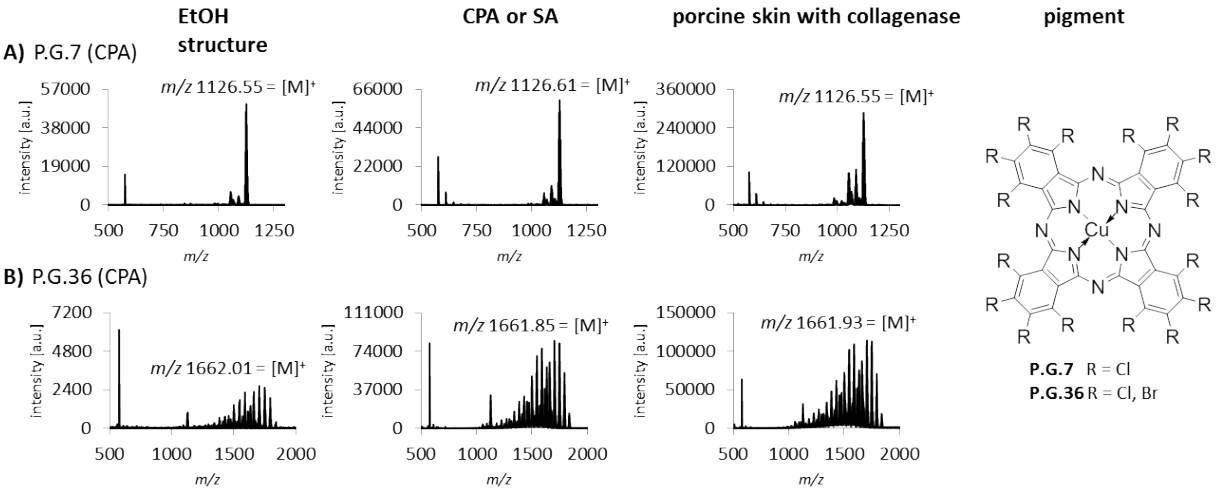
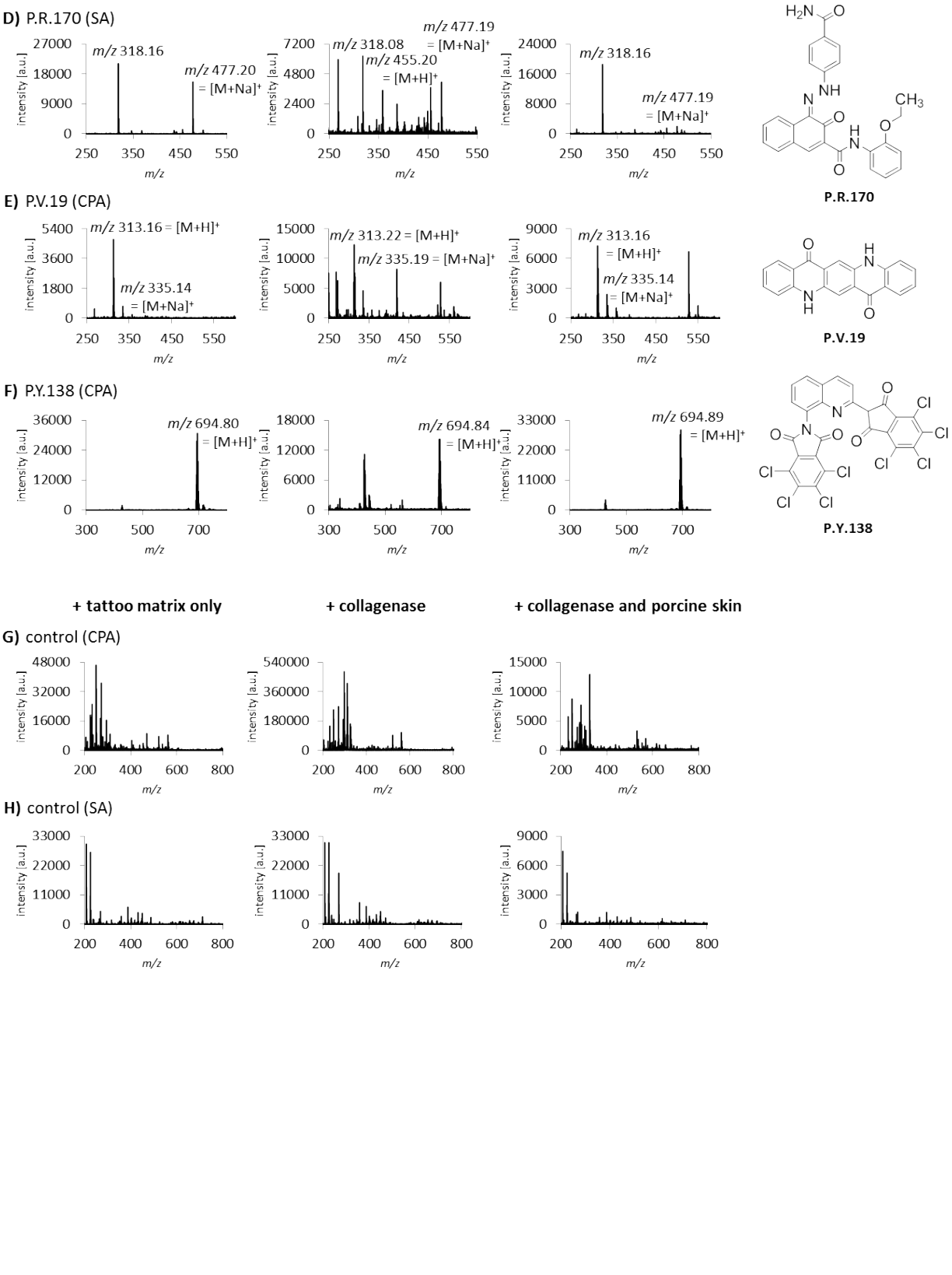


Fig. S2 Comparison of spectra of pure pigments dispersed in the tattoo matrix and pigments extracted from porcine skin. Optimized preparation of biological samples included enzymatic digestion with collagenase followed by mechanical disruption. Spectra of pure pigments in EtOH (left) or matrix (middle) are displayed for each pigment in A)-F), respectively. Spectra of pigments extracted from pig skin are shown on the right. The matrix used for each pigment is given in brackets. B) In P.G.36, the number of chlorine and bromine substituents may vary due to its kind of manufacturing process. Here, the labeled peak corresponds to $C_{32}Br_{12}Cl_4CuN_8$. G), H) Control spectra of tattoo matrix, collagenase and collagenase treated porcine skin in CPA and SA matrix, respectively.




D) P.R.170 (SA)
m/z 318.16
m/z 477.20 = [M+Na]+
m/z 318.08
m/z 455.20 = [M+H]+
m/z 477.19 = [M+Na]+
m/z 318.16
m/z 477.19 = [M+Na]+
intensity [a.u.]
m/z
P.R.170
E) P.V.19 (CPA)
m/z 313.16 = [M+H]+
m/z 335.14 = [M+Na]+
m/z 313.22 = [M+H]+
m/z 335.19 = [M+Na]+
m/z 313.16 = [M+H]+
m/z 335.14 = [M+Na]+
P.V.19
F) P.Y.138 (CPA)
m/z 694.80 = [M+H]+
m/z 694.84 = [M+H]+
m/z 694.89 = [M+H]+
P.Y.138
+ tattoo matrix only
+ collagenase
+ collagenase and porcine skin
G) control (CPA)
H) control (SA)