CHAPTER 5

CONVERGENCE AND UNIQUENESS
ANALYSIS OF NMF ON THE SEPARATION OF
OVERLAPPED CHROMATOGRAMS

5.1 INTRODUCTION

The NMF algorithms, namely NMFmse, NMFdiv and sNMF have been modified and used as M-NMF algorithms for chromatogram separation so far in this work. But, there is no convergence of those algorithms to a stable limit point [62-64] and no uniqueness in results due to random initialization. To get convergence to a stable limit point, the multiplicative update [37] has been modified [62]. To get uniqueness in results, the use of three different initialization methods, namely EFA [31], NNDSVD based initialization [73] and Robust (ALS-NMF) [75] initialization has also been used in the proposed algorithm, instead of random initialization. The proposed ML-NMF (Modified M-NMF) algorithms namely ML-NMFdiv (Modified M-NMFdiv), ML-sNMF (Modified M-sNMF) and ML-NMFmse (Modified M-NMFmse), which have been incorporated with the above two modifications, are applied on the simulated and experimental overlapped chromatograms of acetone and acrolein mixture to resolve the chromatograms and the analyzed results are described in this chapter. The results of proposed ML-NMF algorithms are compared with the results of existing Multivariate Curve Resolution-Alternating Least Square (MCR-ALS) method.
5.2 CONVERGENCE AND UNIQUENESS ISSUES OF NMF

5.2.1 Uniqueness of NMF algorithm

Several runs of the NMF algorithm with random initialization will give different answers which result with non-uniqueness. Hence, it is important to have efficient and consistent initial matrices $W$ and/or $H$ because the solution and convergence provided by NMF algorithms highly depend on initial conditions. To speed up the convergence and to get unique results, Robust (ALS-NMF), NNDSVD and EFA based initializations strategies have been used in this proposed algorithm for the overlapped chromatogram separation.

5.2.2 Convergence of NMF algorithm

The multiplicative updates of NMF algorithm [37] do not assure convergence to a stationary point [62, 63-67]. There are difficulties exist in the multiplicative algorithm due to zero values in the matrices [62].

5.3 PROPOSED ML-NMF ALGORITHMS

In the proposed ML-NMF algorithms, the multiplicative update rules of M-NMF algorithms used so far in this work have been replaced by the modified update to improve the convergence to a stable limit point. Three initialization strategies have also been used in the proposed ML-NMF algorithms to get uniqueness in results with additional constraints. The ML-NMFdiv, ML-sNMF and ML-NMFmse algorithms have been proposed for overlapped chromatograms separation in this work.

5.3.1 Proposed ML-NMFdiv algorithm

The multiplicative update of M-NMFdiv algorithm proposed in this work has been modified and proposed as ML-NMFdiv for the convergence of this algorithm to
a stable limit point. To get unique results, NNDSVD [73], EFA [31] and Robust initialization [75] methods are also used.

The proposed ML-NMFDiv algorithm has the following objective function which has to be minimized:

\[
\text{Objective function } (f) = \sum_{i=1}^{m} \sum_{j=1}^{n} \left[ A_{ij} \log \left( \frac{A_{ij}}{(WH)_{ij}} \right) \cdot A + WH \right]
\]  

(5.1)

Assign \( \varepsilon > 0 \) and \( \delta > 0 \)

i) Initialize \( W_{iq}^I \geq 0 \) and \( H_{ij}^I \geq 0, \forall i, q, j \), using Robust initialization or NNDSVD or EFA.

ii) Let \( p \) be the number of iterations

For \( k = 1, 2, \ldots p \)

a) If \((W^k, H^k)\) is stationary, stop.

Else

\[
H^{k,n}_{qj} = H^k_{qj} - \frac{\tilde{H}^k_{qj}}{((W^k)^T W^k H^k)_{qj} + \varepsilon} \nabla_H f(W^k, H^k)_{qj}, \forall q, j
\]

(5.2)

\[
W^{k,n}_{iq} = W^k_{iq} - \frac{\tilde{W}^k_{iq}}{(\tilde{W}^k H^{k,n}(H^{k,n})^T)_{iq} + \varepsilon} \nabla_W f(W^k, H^{k,n})_{iq}, \forall i, q
\]

(5.3)

where

\[
\nabla_W f(W, H) = (WH - A)H^T \text{ and } \nabla_H f(W, H) = W^T (WH - A)
\]

\[
\tilde{H}^k_{qj} / ((W^k)^T W^k H^k)_{qj} + \varepsilon \]

is the step size.
where

$$
\overline{H}_{qj}^k \equiv \begin{cases} 
H_{qj}^k & \text{if } \nabla_H f(W^k, H^k)_{qj} \geq 0, \\
\max(H_{qj}^k, \delta) & \text{if } \nabla_H f(W^k, H^k)_{qj} < 0
\end{cases}
$$

$\varepsilon$ [77, 78] and $\delta$ are the small positive numbers assumed to avoid division by zero.

b) Normalize $W^{k,n}$ and $H^{k,n}$ to $H^{k+1}$ and $W^{k+1}$ respectively so that $W^{k+1}$'s column sum is one.

c) Unimodality and selectivity constraints are additionally imposed into the algorithm so as to use the algorithm for chromatogram separation.

d) The iterative algorithm used has been stopped when the number of iterations $p$ achieves or exceeds a predefined maximum number of iteration. It is assumed that there is no need for peak shifting, base line correction and noise filtering.

iii) Calculate the objective function using new updated $W$ and $H$.
iv) Repeat from (iii) to (iv) until convergence is achieved.

The modified multiplicative update overcomes the difficulties exist in the convergence of M-NMFdiv algorithm. Element wise operation has been carried out in the iterative steps. Each column of $W$ matrix gives the chromatogram of individual chemical present in the mixture.

5.3.1.1 Simulation study using proposed ML-NMFdiv algorithm with EFA based initialization

In the first case, the proposed ML-NMFdiv algorithm has been initialized with EFA based estimate and applied on the simulated chromatogram of acetone and acrolein mixture.
The application of proposed ML-NMFdiv algorithm (EFA based initialization) on the partially overlapped chromatogram resolves the individual components as shown in Fig.5.1. The resolved acetone is similar to its standard and has correlation coefficient and signal recovery of 0.9903 and 17.1473 dB respectively. The resolved acrolein has more similarity with its standard and has correlation coefficient of 0.9999 and signal recovery of 37.6693 dB (Table 5.1 & 5.2). The resolved components elute exactly at the same time as that of the standards. Fig.5.2 shows the relation between objective function and number of iterations. The objective function has an initial value of 234151.24554 and finally converges to 0.00001.

The proposed ML-NMFdiv algorithm (EFA based initialization) has been tested on the severely overlapped chromatogram and the results are shown in Fig.5.3.

![Fig.5.1](image)

**Fig.5.1** Unresolved (partially overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFdiv algorithm with EFA based initialization
Fig. 5.2 Objective function vs. Number of iterations for proposed ML-NMFdiv implementation (EFA based initialization) on partially overlapped chromatogram separation

Fig. 5.3 Unresolved (severely overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFdiv algorithm with EFA based initialization

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The resolved acetone and acrolein elute exactly at the same time as that of the standards. It gives correlation coefficients and signal recoveries of the resolved acetone and acrolein as 0.9999, 0.9943 and 39.1874 dB, 20.2173 dB respectively (Table 5.3 & 5.4). Fig.5.4 shows the minimization of Objective function vs. Number of iterations for proposed ML-NMFdiv on severely overlapped chromatogram separation. The initial value of the objective function is 67963.06872 and it converges to 0.00012.

![Objective function vs. Number of iterations for proposed ML-NMFdiv implementation (EFA based initialization) on severely overlapped chromatogram](image)

The embedded chromatogram of acetone and acrolein mixture are then resolved with proposed ML-NMFdiv algorithm (EFA based initialization). The resolved chromatograms are shown in Fig.5.5. The shape of acetone largely deviates from its standard and has very poor correlation coefficient and signal recovery which are 0.6914 and 2.6756 dB respectively. The peak retention times of resolved acetone and acrolein are exactly same as that of the standards. The resolved acrolein has correlation coefficient of 0.9998 and signal recovery of 34.8283 dB respectively.
The objective function converges from 2064.52307 to 0.0001 as shown in Fig.5.6.

Fig.5.5 Unresolved (embedded) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFdiv with EFA based initialization

Fig.5.6 Objective function vs. Number of iterations for proposed ML-NMFdiv implementation (EFA based initialization) on embedded chromatogram separation
5.3.1.2 Simulation study using proposed ML-NMFdiv algorithm with NNDSVD based initialization

In the second case, the proposed ML-NMFdiv algorithm has been initialized with NNDSVD method and applied on the overlapped chromatograms for separation.

The proposed ML-NMFdiv algorithm has been initialized with NNDSVD method and applied on the partially overlapped chromatogram for separation. The unresolved and the resolved chromatograms are shown in Fig. 5.7. The acetone resolves with a correlation coefficient of 1.0000 and signal recovery of 50.7955 dB. The acrolein resolves with a correlation coefficient of 0.9999 and signal recovery of 37.9009 dB (Table 5.1 & 5.2). The resolved components elute at exactly the same time as that of their standards. The convergence of proposed ML-NMFdiv on partially overlapped chromatogram separation is shown in Fig. 5.8. The initial value of the objective function is 5216.01210 and it converges to 0.00003.

![Fig. 5.7 Unresolved (partially overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFdiv algorithm with NNDSVD based initialization](image-url)
The proposed ML-NMFdiv algorithm with NNDSVD based initialization is then tested with the severely overlapped chromatogram. The resolved chromatograms are shown in Fig.5.9. The resolved acetone and acrolein have the correlation coefficients as 0.9998, 0.9943 and signal recoveries as 34.9435 dB, 20.2159 dB respectively (Table 5.3 & 5.4). Fig.5.10 shows the convergence of this algorithm with respect to the number of iterations. The objective function begins with 1581.95424 and finally it converges to 0.00035.

Then the embedded chromatogram of acetone and acrolein mixture is resolved using proposed ML-NMFdiv with NNDSVD based initialization and the results are shown in Fig.5.11. The resolved acetone elutes at 1.127min but the shape is not identical with its standard and has poor correlation coefficient and signal recovery, which are 0.5963 and 1.4826 dB respectively. The resolved acrolein elute at 1.187min and has correlation coefficient of 0.9526 and signal recovery of 10.5284 dB (Table 5.5 & 5.6). Fig.5.12 shows the Objective function vs. Number of iterations for

**Fig.5.8 Objective function vs. Number of iterations for proposed ML-NMFdiv implementation (NNDSVD based initialization) on partially overlapped chromatogram separation**
proposed ML-NMFdiv on embedded chromatogram separation. The initial value of the objective function is $1130.23888$ and it converges to $0.00005$.

**Fig. 5.9** Unresolved (severely overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFdiv algorithm with NNDSVD based initialization

**Fig. 5.10** Objective function vs. Number of iterations for proposed ML-NMFdiv implementation (NNDSVD based initialization) on severely overlapped chromatogram
Fig. 5.11 Unresolved (embedded) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFdiv algorithm with NNDSVD based initialization

Fig. 5.12 Objective function vs. Number of iterations for proposed ML-NMFdiv implementation (NNDSVD based initialization) on embedded chromatogram
5.3.1.3 Simulation study using proposed ML-NMFdiv algorithm with Robust initialization

In the third case, the proposed ML-NMFdiv algorithm has been initialized with Robust initialization method and applied on the overlapped chromatograms of acetone and acrolein mixture to resolve the individual chromatogram.

The proposed ML-NMFdiv algorithm with Robust initialization has been applied on the partially overlapped chromatogram. The resolved chromatograms are identical with that of their standards as shown in Fig.5.13. They have correlation coefficients as 1.0000, 0.9999 and signal recoveries as 48.5021 dB, 37.6310 dB for acetone and acrolein respectively (Table 5.1 & 5.2). The convergence of this algorithm is shown in Fig.5.14, which shows the convergence from 146.37315 to 0.00004.

Fig.5.13 Unresolved (partially overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFdiv algorithm with Robust initialization
Then the proposed ML-NMFdiv with Robust initialization has been applied on the severely overlapped chromatogram to resolve the individual chromatogram. The results are shown in Fig.5.15. The correlation coefficients and signal recoveries are 0.9997, 0.9943 and 32.9232 dB, 20.2147 dB for acetone and acrolein respectively (Table 5.3 & 5.4). Fig.5.16 shows the convergence of the proposed algorithm from 19.55333 to 0.00058.

The embedded chromatogram is then resolved with proposed ML-NMFdiv with Robust initialization. The resolved acetone shown in Fig.5.17 has the correlation coefficient of 0.9993 and signal recovery of 29.1994 dB. The resolved acrolein shown in the same figure has the correlation coefficient of 0.9563 and signal recovery of 10.8769 dB (Table 5.5 & 5.6). Fig.5.18 shows the convergence trend of proposed ML-NMFdiv on embedded chromatogram separation. The algorithm starts with a minimum value of 26.81801 and finally converges to 0.00002.

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**Fig.5.14** Objective function vs. Number of iterations for proposed ML-NMFdiv implementation (Robust initialization) on partially overlapped chromatogram separation
Fig. 5.15 Unresolved (severely overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFdiv algorithm with Robust initialization.

Fig. 5.16 Objective function vs. Number of iterations for proposed ML-NMFdiv implementation (Robust initialization) on severely overlapped chromatogram separation.
Fig. 5.17 Unresolved (embedded) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFdiv algorithm with Robust initialization

Fig. 5.18 Objective function vs. Number of iterations for proposed ML-NMFdiv implementation (Robust initialization) on embedded chromatogram separation
5.3.2 Proposed ML-sNMF algorithm

The objective function of ML-sNMF has been defined by using the divergence function between \( A \) and \( WH \) along with sparse constraint, given as

\[
\text{Objective function}(f) = \sum_{i=1}^{m} \sum_{j=1}^{n} (A_{ij} \log \left( \frac{A_{ij}}{(WH)_{ij}} \right) - A_{ij} + WH) + \alpha \sum_{q=1}^{k} \sum_{j=1}^{n} H_{qj}
\]

(5.4)

where \( \alpha \) was assumed a positive constant.

A sparse solution to the above constrained minimization can be found by the following update rules of \( W \) and \( H \):

i) Assign \( \varepsilon > 0 \) and \( \delta > 0 \)

ii) Initialize \( W_{iq}^1 \geq 0 \) and \( H_{qj}^1 \geq 0, \forall i, q, j \), using Robust initialization or NNDSVD or EFA.

iii) Let \( p \) be the number of iterations.

For \( k = 1, 2 \ldots p \)

a) If \( (W^k, H^k) \) is stationary, stop.

Else

\[
H_{qi}^{kn} = H_{qi}^k - \frac{\tilde{H}_{qi}^k}{((W^k)^TW^kH^k)_{qi} + \varepsilon} \nabla_{H} f(W^k, H^k)_{qj}, \forall q, j
\]

(5.5)

\[
W_{iq}^{kn} = W_{iq}^k - \frac{\tilde{W}_{iq}^k}{(W^kH^{kn}(H^{kn})^T)_{iq} + \varepsilon} \nabla_{W} f(W^k, H^{kn})_{qj}, \forall i, q
\]

(5.6)

where

\[
\nabla_{w} f(W, H) = (WH - A)H^T \text{ and } \nabla_{H} f(W, H) = W^T(WH - A).
\]
\[
\frac{\tilde{H}_{qj}^k}{((W^k)^T W^k \tilde{H}^k)_{qj} + \varepsilon}
\]
is the step size

where

\[
\tilde{H}_{qj}^k = \begin{cases} H_{qj}^k & \text{if } \nabla_{H} f(W^k, H^k)_{qj} \geq 0, \\ \max(H_{qj}^k, \delta) & \text{if } \nabla_{H} f(W^k, H^k)_{qj} < 0 \end{cases}
\]

\(\varepsilon\) [77,78] and \(\delta\) are the small positive numbers assumed to avoid division by zero.

b) Normalize \(W^{k,n}\) and \(H^{k,n}\) to \(H^{k+1}\) and \(W^{k+1}\) respectively so that \(W^{k+1}\)'s column sum is one.

c) Unimodality and selectivity constraints are additionally imposed into the algorithm so as to use the algorithm for chromatogram separation.

d) The iterative algorithm used has been stopped when the number of iterations \(p\) achieves or exceed a predefined maximum number of iteration. It is assumed that there is no need for peak shifting, baseline correction and noise filtering.

iv) Calculate the objective function using new updated \(W\) and \(H\).

v) Repeat from (iii) to (iv) until convergence is achieved.

The modified multiplicative update overcomes the difficulties that exist in the convergence of M-sNMF algorithm. Each column of \(W\) matrix gives the chromatogram of individual chemical present in the mixture. The algorithm can handle sparseness in the data. The maximum number of iterations has to be specified.
5.3.2.1 Simulation study using proposed ML-sNMF algorithm with EFA based initialization

Initially, the proposed ML-sNMF algorithm has been initialized with EFA method and then applied for resolving the overlapped chromatograms. On account of applying ML-sNMF algorithm (EFA based initialization) over the simulated partially overlapped chromatogram, it determines the individual chromatogram separately as illustrated in Fig.5.19. The shape of resolved acetone has good matching with its standard. Accordingly, the resolved chromatograms are compared with the standard chromatograms to get the correlation coefficient between two components and obtained as 1.0000 and 0.9993 for acetone and acrolein respectively. Meanwhile the signal recoveries of the acetone and acrolein are 51.6052 dB and 29.0729 dB respectively (Table 5.1 & 5.2). The Objective function vs. Number of iterations for the suggested ML-sNMF on partially overlapped chromatogram separation is shown in Fig.5.20. The objective function begins with 234151.24582 and gets converged to 0.00006.

![Fig.5.19 Unresolved (partially overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-sNMF algorithm with EFA based initialization](image)

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Fig.5.20 Objective function vs. Number of iterations for proposed ML-sNMF implementation (EFA based initialization) on partially overlapped chromatogram separation

Resolution of the severely overlapped chromatogram has been done as shown in Fig.5.21 by employing proposed ML-sNMF algorithm (EFA based initialization). The comparative analysis of the results with the standards gives 0.9999 and 39.7917 dB as the correlation coefficient and signal recovery of acetone and 0.9943 and 20.2171 dB as the correlation coefficient and signal recovery of acrolein (Table 5.3 & 5.4). The convergence of this proposed algorithm is given in Fig.5.22 which shows the convergence from 67963.06968 to 0.00000.

The proposed ML-sNMF algorithm (EFA based initialization) is then applied on the simulated embedded chromatogram and the chromatograms are separated individually. The unresolved and resolved chromatograms are as shown in Fig.5.23. By relating resolved chromatograms with the standard chromatograms, it procure the correlation coefficients for the acetone and acrolein as 0.5495 and 0.9940 respectively. Consequently, the signal recoveries are 0.8856 dB and 19.4033 dB respectively (Table 5.5 & 5.6). The very poor signal recovery of acetone is due to the mismatch of its shape with that of the standard. Fig.5.24 depicts the minimization of objective function with respect to number of iterations for the proposed ML-sNMF on
embedded chromatogram separation. The algorithm converges from 2064.52347 to 0.02230.

Fig. 5.21 Unresolved (severely overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-sNMF algorithm with EFA based initialization

Fig. 5.22 Objective function vs. Number of iterations for proposed ML-sNMF implementation (EFA based initialization) on severely overlapped chromatogram separation
Fig. 5.23 Unresolved (embedded) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-sNMF algorithm with EFA based initialization

Fig. 5.24 Objective function vs. Number of iterations for proposed ML-sNMF implementation (EFA based initialization) on embedded chromatogram separation
5.3.2.2 Simulation study using proposed ML-sNMF algorithm with NNDSVD based initialization

Secondly, the proposed ML-sNMF algorithm with NNDSVD based initialization has been used for overlapped chromatogram separation.

The proposed ML-sNMF algorithm (NNDSVD based initialization) is applied on the simulated partially overlapped chromatogram of acetone and acrolein mixture. The Fig.5.25 shows the resolved chromatograms. The signal recovery and correlation coefficient of acetone are 51.6790 dB and 1.0000 and this result is found to be comparatively higher than those of acrolein, which are 37.8519 dB and 0.9999 (Table 5.1 & 5.2). Fig.5.26 shows the relation between objective function and number of iterations. The algorithm converges from 5216.01750 to -0.00001.

Fig.5.25 Unresolved (partially overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-sNMF algorithm with NNDSVD based initialization
In the case of using proposed ML-sNMF algorithm (NNDSVD based initialization) over the simulated severely overlapped chromatogram, it separates the individual chromatogram. The results obtained are shown in Fig.5.27. The correlation coefficient has been acquired by comparing resolved chromatograms with the standard chromatograms. As a result, the correlation coefficients are 0.9999 and 0.9943 for acetone and acrolein respectively. Furthermore the signal recoveries are 36.2357 dB and 20.2163 dB for acetone and acrolein respectively (Table 5.3 & 5.4). The minimization of Objective function vs. Number of iterations for the proposed ML-sNMF on embedded chromatogram separation is shown in Fig.5.28. The algorithm gets converged from 1581.95681 to 0.00015. Minimization of objective function vs. Number of iterations for the proposed ML-sNMF on embedded chromatogram separation is shown in Fig.5.28. The algorithm converges from 1581.95681 to 0.00015.
Fig.5.27 Unresolved (severely overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-sNMF algorithm with NNDSVD based initialization

Fig.5.28 Objective function vs. Number of iterations for proposed ML-sNMF implementation (NNDSVD based initialization) on severely overlapped chromatogram separation
On applying the proposed ML-sNMF algorithm (NNDSVD based initialization) to the simulated embedded chromatogram of acetone and acroleine, Fig.5.29

Fig.5.29 Unresolved (embedded) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-sNMF algorithm with NNDSVD based initialization

Fig.5.30 Objective function vs. Number of iterations for proposed ML-sNMF implementation (NNDSVD based initialization) on embedded chromatogram separation
Individual chromatogram is resolved as shown in Fig.5.29. The correlation coefficients are 0.6206 and 0.9376 and signal recoveries are 1.4883 dB and 9.6967 dB for acetone and acrolein respectively (Table 5.5 & 5.6). Fig.5.30 shows the convergence of the proposed algorithm from 25.70305 to 0.03095.

5.3.2.3 Simulation study using proposed ML-sNMF algorithm with Robust initialization

In the third case, the proposed ML-sNMF algorithm has been initialized with Robust initialization method and applied on overlapped chromatograms for separation.

The proposed ML-sNMF algorithm (Robust initialization) has been used to separate the individual chromatogram from the simulated partially overlapped chromatogram as described in Fig.5.31. The resolved chromatograms are compared with the standard chromatograms; the correlation coefficients of acetone and acrolein

![Fig.5.31 Unresolved (partially overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-sNMF algorithm with Robust initialization](image-url)
are obtained as 1.0000, 1.0000 respectively. Similarly, the signal recoveries of those components are 49.4276 dB, 49.6969 dB respectively (Table 5.1 & 5.2). The objective function vs. Number of iterations for the proposed ML-sNMF on partially overlapped chromatogram separation is as shown in Fig.5.32 and it converges from 146.37342 to -0.00001.

In order to resolve the individual chromatogram from the simulated severely overlapped chromatogram of acetone and acrolein, the proposed ML-sNMF algorithm (Robust initialization) has been used. The resolved individual peaks are shown in Fig.5.33. The values of correlation coefficient and signal recovery of acetone are 0.9998 and 34.6064dB respectively (Table 5.3 & 5.4). The values of correlation coefficient and signal recovery of acrolein are 0.9943 and 20.2154dB respectively. The minimization of objective function in different iterations is shown in Fig.5.34 that shows the convergence from 19.55430 to 0.00027.

![Objective function vs. Number of iterations for proposed ML-sNMF implementation (Robust initialization) on partially overlapped chromatogram separation](image)
The proposed ML-sNMF algorithm (Robust initialization) is used to resolve the individual chromatogram from the simulated embedded chromatogram of acetone and acrolein mixture. The resolved components are shown in the Fig.5.35. On comparison of the result of proposed ML-sNMF with the available standards, the correlation coefficient and signal recovery of acetone are found to be 0.9993 and 29.2041 dB respectively (Table 5.5 & 5.6). On similar comparison, the values for acrolein are found to be 0.9563 and 10.8768 dB. The convergence of the objective function for the proposed algorithm on chromatogram separation, which varies from 26.81840 to -0.00006, is shown in Fig.5.36.
Fig. 5.34 Objective function vs. Number of iterations for proposed ML-sNMF implementation (Robust initialization) on severely overlapped chromatogram separation.

Fig. 5.35 Unresolved (embedded) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-sNMF algorithm with Robust initialization.
5.3.3 Proposed ML-NMFmse algorithm

The objective function of ML-NMFmse has been defined using the Euclidean distance between $A$ and $WH$ which has to be minimized, subject to the constraints $W, H \geq 0$.

$$\text{Objective function } (f) = 0.5 \sum_{i=1}^{m} \sum_{j=1}^{n} ((A - (WH))_{ij})^2$$  \hspace{1cm} (5.7)

An iterative approach to reach a local minimum of this objective function is given by the following multiplicative update rules of $W$ and $H$:

i) Assign $\varepsilon > 0$ and $\delta > 0$

ii) Initialize $W_{iq}^1 \geq 0$ and $H_{aq}^1 \geq 0, \forall i, q, j$, using Robust initialization or NNDSVD or EFA.

iii) Let $p$ be the number of iterations.
For $k = 1, 2 \ldots p$

a) If $(W^k, H^k)$ is stationary, stop.

Else

$$H_{k,n}^{ij} = H_{ij}^k \frac{\bar{H}_{ij}^k}{((W^k)^T W^k H^k)_{ij} + \varepsilon} \nabla_H f(W^k, H^k)_{ij}, \forall q, j \quad (5.8)$$

$$W_{k,n}^{ij} = W_{ij}^k \frac{\bar{W}_{ij}^k}{(W^k H^{k,n})^T_{ij} + \varepsilon} \nabla_W f(W^k, H^{k,n})_{ij}, \forall i, q \quad (5.9)$$

where

$$\nabla_W f(W, H) = (WH - A)H^T \quad \text{and} \quad \nabla_H f(W, H) = W^T (WH - A) .$$

$$\frac{\bar{H}_{ij}^k}{((W^k)^T W^k \bar{H}^k)_{ij} + \varepsilon}$$

is the step size

where

$$\bar{H}_{ij}^k \equiv \begin{cases} H_{ij}^k & \text{if} \quad \nabla_H f(W^k, H^k)_{ij} \geq 0, \\ \max(H_{ij}^k, \delta) & \text{if} \quad \nabla_H f(W^k, H^k)_{ij} < 0 \end{cases}$$

$\varepsilon$ [77, 78] and $\delta$ are the small positive numbers assumed to avoid division by zero.

b) Normalize $W^{k,n}$ and $H^{k,n}$ to $H^{k+1}$ and $W^{k+1}$ respectively so that $W^{k+1}$'s column sum is one.

c) Unimodality and selectivity constraints are additionally imposed into the algorithm so as to use the algorithm for chromatogram separation.
d) The iterative algorithm used has been stopped when the number of iterations \( p \) achieves or exceeds a predefined maximum number of iteration. It is assumed that there is no need for peak shifting, baseline correction and noise filtering.

iv) Calculate the objective function using new updated \( W \) and \( H \).

v) Repeat from step (iii) to (iv) until convergence is achieved.

The modified multiplicative update overcomes the difficulties exist in the convergence of M-NMFmse algorithm. Each column of \( W \) matrix gives the chromatogram of individual chemical present in the mixture. The maximum number of iterations has to be specified.

5.3.3.1 Simulation study using proposed ML-NMFmse algorithm with EFA based initialization

Initially, the EFA based initialization has been used to initialize the matrices of the proposed ML-NMFmse algorithm and to resolve the overlapped chromatograms.

To resolve the individual components from the simulated partially overlapped chromatogram of acetone and acroline, the proposed ML-NMFmse algorithm with EFA based initialization has been applied. The resolved components are shown in the Fig.5.37, in which their shapes are similar with its standard. The correlation coefficient and signal recovery of acetone are found to be 1.0000 and 52.1665 dB respectively. The correlation coefficient and signal recovery of acroline are found to be 0.9967 and 22.1431 dB respectively (Table 5.1 & 5.2). Fig.5.38 shows the objective function vs. Number of iterations for proposed ML-NMFmse implementation on chromatogram separation. The iteration begins with a value of 894123220 and finally converged to 0.00000.
Unresolved (partially overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFmse algorithm with EFA based initialization.

Objective function vs. Number of iterations for proposed ML-NMFmse implementation (EFA based initialization) on partially overlapped chromatogram separation.
The simulated severely overlapped chromatogram of acetone and acroline mixture has been subjected to proposed ML-NMFmse algorithm with EFA estimate as initial matrices and the individual components are resolved successfully as shown in Fig.5.39. The elution time of individually resolved components are same as that of their standards. The correlation coefficient and signal recovery of acetone are found as 0.9999 and 39.7917dB and the correlation coefficient and signal recovery of acroline are found as 0.9943 and 20.2171dB (Table 5.3 & 5.4). The convergence of objective function for the proposed algorithm is shown in Fig.5.40, which starts with a value of 23022193 and converges to 0.00000.

On applying the proposed ML-NMFmse algorithm (EFA estimate as initial matrices) to the simulated embedded chromatogram, the individual components are resolved and given in Fig.5.41. Acetone’s correlation coefficient and signal recovery are found to be 0.6915 and 2.6768dB. The correlation coefficient and signal recovery of acroline are found to be 0.9998 and 34.8281dB (Table 5.5 & 5.6). Fig.5.42 shows the trend of the objective function for different iterations that converges from 14131 to 0.00003.

![Unresolved and Resolved Chromatograms](image)

**Fig.5.39** Unresolved (severely overlapped) chromatogram of acetone and acroline mixture and resolved chromatograms using proposed ML-NMFmse algorithm with EFA based initialization
Fig. 5.40 Objective function vs. Number of iterations for proposed ML-NMFmse implementation (EFA based initialization) on severely overlapped chromatogram separation

Fig. 5.41 Unresolved (embedded) chromatogram of acetone and acrolein mixture and resolved chromatograms using ML-NMFmse with EFA based initialization
5.3.3.2 Simulation study using proposed ML-NMFmse algorithm with NNDSVD based initialization

The proposed ML-NMFmse algorithm has been initialized with NNDSVD method and used for overlapped chromatogram separation.

The proposed ML-NMFmse algorithm has been initialized with NNDSVD method and applied on the partially overlapped chromatogram for separation. The unresolved and the resolved chromatograms are shown in Fig.5.43. The resolved acetone has correlation coefficient of 1.0000 and signal recovery of 52.1992dB. The resolved acrolein has a correlation coefficient of 0.9967 and signal recovery of 22.1431dB (Table 5.1 & 5.2). The convergence of objective function for the proposed ML-NMFmse on partially overlapped chromatogram separation is shown in Fig.5.44. The initial value of the objective function is 139366 and it is converged to 0.00000.
Fig. 5.43 Unresolved (partially overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFMSE with NNDSVD based initialization.

Fig. 5.44 Objective function vs. Number of iterations for proposed ML-NMFMSE implementation (NNDSVD based initialization) on partially overlapped chromatogram separation.
The proposed ML-NMFmse algorithm with NNDSVD based initialization is then tested with the severely overlapped chromatogram. The resolved chromatograms are shown in Fig. 5.45. The resolved chromatograms are similar to their standards and have correlation coefficients of 0.9999, 0.9943 and signal recoveries of 36.2225dB, 20.2163dB for acetone and acrolein respectively (Table 5.3 & 5.4). Fig. 5.46 shows the minimization of Objective function vs. Number of iterations for proposed ML-NMFmse on severely overlapped chromatogram separation. The initial value of the objective function is 11588 and it gets converged to 0.00001.

The proposed ML-NMFmse algorithm (NNDSVD based initialization) is then applied on the simulated embedded chromatogram of acetone and acrolein mixture. The Fig. 5.47 shows the resolved components. The signal recovery and correlation coefficient of acetone are 1.4826 dB and 0.5963 and this result is found to be comparatively lesser than those of acrolein, which are 10.5284dB and 0.9526 (Table 5.5 & 5.6). Fig. 5.48 shows about the convergence of objective function and number of iterations. The algorithm converges from 6448 to 0.00001.

![Fig.5.45 Unresolved (severely overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFmse with NNDSVD based initialization](image)
Fig. 5.46  Objective function vs. Number of iterations for proposed ML-NMFmse implementation (NNDSVD based initialization) on severely overlapped chromatogram separation

Fig. 5.47  Unresolved (embedded) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFmse with NNDSVD based initialization
5.3.3.3 Simulation study using proposed ML-NMFmse algorithm with Robust initialization

In the third case, the proposed ML-NMFmse algorithm has been initialized with Robust initialization and used for overlapped chromatogram separation.

The proposed ML-NMFmse algorithm (Robust initialization) has been used to separate partially overlapped chromatogram and the result is shown in Fig.5.49. The resolved chromatograms are compared with the standard chromatograms. The correlation coefficient of acetone and acrolein are found to be 1.0000 and 0.9999 respectively. Similarly, the signal recoveries of those components are 49.7770 dB and 37.6111 dB respectively (Table 5.1 & 5.2). The convergence of Objective function vs. Number of iterations for proposed ML-NMFmse on partially overlapped chromatogram separation is as shown in Fig.5.50 and it converges from 548 to 0.00000.

![Objective function vs. Number of iterations for proposed ML-NMFmse implementation (NNDSVD based initialization) on embedded chromatogram separation](image)
Fig. 5.49 Unresolved (partially overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFmse with Robust initialization

Fig. 5.50 Objective function vs. Number of iterations for proposed ML-NMFmse implementation (Robust initialization) on partially overlapped chromatogram separation
Then the use of proposed ML-NMFmse algorithm (Robust initialization) on the chromatogram separation has been tested with the severely overlapped chromatogram and the results are shown in Fig.5.51. The resolved acetone and acrolein are same as that of the standards. It gives correlation coefficients as 0.9998, 0.9943 and signal recoveries as 34.5066 dB, 20.2153 dB for the resolved acetone and acrolein respectively (Table 5.3 & 5.4). Fig.5.52 shows the Objective function vs. Number of iterations for proposed ML-NMFmse on severely overlapped chromatogram separation. The initial value of the objective function is 4750.68638 and it is converged to 0.00001.

Resolution of the embedded chromatogram has also been done as shown in Fig.5.53 by employing proposed ML-NMFmse algorithm (Robust initialization). The comparative analysis of the results with the standards gives 0.9993 and 29.1994 dB as the correlation coefficient and signal recovery of acetone and 0.9563 and 10.8769 dB as the correlation coefficient and signal recovery of acrolein (Table 5.5 & 5.6). The convergence of objective function for the proposed algorithm is shown in Fig.5.54, which converges from 3.84494 to 0.00000.

![Fig.5.51 Unresolved (severely overlapped) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFmse with Robust initialization](image-url)
**Fig. 5.52** Objective function vs. Number of iterations for proposed ML-NMFmse implementation (Robust initialization) on severely overlapped chromatogram separation.

**Fig. 5.53** Unresolved (embedded) chromatogram of acetone and acrolein mixture and resolved chromatograms using proposed ML-NMFmse with Robust initialization.
All the proposed ML-NMF algorithms in this work for chromatographic resolution are effective. When chromatographic profiles of the mixture of two components of similar peak-height and almost of the same retention time are analysed, it is quite a troublesome issue for analysts. But the solutions of ML-NMF algorithms are reasonable and acceptable. The ML-NMFmse algorithm requires less additional constraints to be imposed and hence its implementation is comparatively very simple. ML-NMFdiv and ML-sNMF requires more additional constraints. All the proposed ML-NMF algorithms in this work render almost similar performance on the simulated overlapped chromatograms separation which is evident from the Table 5.1 to 5.6. The mean value of the performance measures are considered for comparison, only if the correlation coefficients of both the resolved components are more than 0.9. Among the comparison, the performances of the proposed methods are comparatively poor than the MCR-ALS method for partially overlapped chromatogram. But the EFA based initialization along with proposed ML-sNMF and ML-NMFmse algorithms give better performance for severely overlapped
chromatogram separation. The proposed ML-sNMF algorithms with Robust method of initialization gives better performance for embedded chromatogram separation.

Table 5.1 Correlation coefficient of resolved components using proposed ML-NMF algorithms and existing MCR-ALS algorithm on the separation of partially overlapped chromatogram

<table>
<thead>
<tr>
<th>Algorithm category</th>
<th>Resolved components</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initialization category</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EFA</td>
</tr>
<tr>
<td>ML-NMFdiv</td>
<td>Resolved acetone</td>
<td>0.9903</td>
</tr>
<tr>
<td></td>
<td>Resolved acrolein</td>
<td>0.9999</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.9951</td>
</tr>
<tr>
<td>ML-sNMF</td>
<td>Resolved acetone</td>
<td>1.0000</td>
</tr>
<tr>
<td></td>
<td>Resolved acrolein</td>
<td>0.9993</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.9997</td>
</tr>
<tr>
<td>ML-NMFmse</td>
<td>Resolved acetone</td>
<td>1.0000</td>
</tr>
<tr>
<td></td>
<td>Resolved acrolein</td>
<td>0.9967</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.9983</td>
</tr>
<tr>
<td>MCR-ALS</td>
<td>Resolved acetone</td>
<td>1.0000</td>
</tr>
<tr>
<td></td>
<td>Resolved acrolein</td>
<td>1.0000</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1.0000</td>
</tr>
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</table>

NB: NA – Not Applicable
Table 5.2 Signal recovery of resolved components using proposed ML-NMF algorithms and existing MCR-ALS algorithm on the separation of partially overlapped chromatogram.

<table>
<thead>
<tr>
<th>Algorithm category</th>
<th>Resolved components</th>
<th>Signal recovery (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initialization category</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
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<td>ML-NMFdiv</td>
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</tr>
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<td></td>
<td>Mean</td>
<td>27.4083</td>
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<tr>
<td>ML-sNMF</td>
<td>Resolved acetone</td>
<td>51.6052</td>
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<tr>
<td></td>
<td>Resolved acrolein</td>
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<tr>
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<td>Mean</td>
<td>40.3391</td>
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<td>Resolved acetone</td>
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<td>Resolved acrolein</td>
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<td>Mean</td>
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<tr>
<td>MCR-ALS</td>
<td>Resolved acetone</td>
<td>49.5348</td>
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<tr>
<td></td>
<td>Resolved acrolein</td>
<td>113.3708</td>
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<td></td>
<td>Mean</td>
<td>81.4528</td>
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NB: NA – Not Applicable
Table 5.3 Correlation coefficient of resolved components using proposed ML-NMF algorithms and existing MCR-ALS algorithm on the separation of severely overlapped chromatogram.

<table>
<thead>
<tr>
<th>Algorithm category</th>
<th>Resolved components</th>
<th>Correlation coefficient</th>
</tr>
</thead>
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<td>Initialization category</td>
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<tr>
<td></td>
<td></td>
<td>EFA</td>
</tr>
<tr>
<td>ML-NMFdiv</td>
<td>Resolved acetone</td>
<td>0.9999</td>
</tr>
<tr>
<td></td>
<td>Resolved acrolein</td>
<td>0.9943</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
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<tr>
<td>ML-sNMF</td>
<td>Resolved acetone</td>
<td>0.9999</td>
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<tr>
<td></td>
<td>Resolved acrolein</td>
<td>0.9943</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.9971</td>
</tr>
<tr>
<td>ML-NMFmse</td>
<td>Resolved acetone</td>
<td>0.9999</td>
</tr>
<tr>
<td></td>
<td>Resolved acrolein</td>
<td>0.9943</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.9971</td>
</tr>
<tr>
<td>MCR-ALS</td>
<td>Resolved acetone</td>
<td>0.9999</td>
</tr>
<tr>
<td></td>
<td>Resolved acrolein</td>
<td>0.9943</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.9971</td>
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</table>

NB: NA – Not Applicable
Table 5.4 Signal recovery of resolved components using proposed ML-NMF algorithms and existing MCR-ALS algorithm on the separation of simulated severely overlapped chromatogram

<table>
<thead>
<tr>
<th>Algorithm category</th>
<th>Resolved components</th>
<th>Signal Recovery (dB)</th>
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</thead>
<tbody>
<tr>
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<td>Initialization category</td>
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<td>ML-NMFdiv</td>
<td>Resolved acetone</td>
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<td>Resolved acrolein</td>
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<td>29.7023</td>
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<td>ML-sNMF</td>
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</tr>
<tr>
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<td>Resolved acrolein</td>
<td>20.2171</td>
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<td>Mean</td>
<td>30.0044</td>
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<td>39.7917</td>
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<td>Resolved acrolein</td>
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</tr>
<tr>
<td></td>
<td>Mean</td>
<td>30.0044</td>
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<td>MCR-ALS</td>
<td>Resolved acetone</td>
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<td>Resolved acrolein</td>
<td>20.3776</td>
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<tr>
<td></td>
<td>Mean</td>
<td>28.1235</td>
</tr>
</tbody>
</table>

NB: NA – Not Applicable
Table 5.5  Correlation coefficient of resolved components using proposed ML-NMF algorithms and existing MCR-ALS algorithm on the separation of embedded chromatogram

<table>
<thead>
<tr>
<th>Algorithm category</th>
<th>Resolved components</th>
<th>Correlation Coefficient</th>
<th>Initialization category</th>
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<td>ML-NMFdiv</td>
<td>Resolved acetone</td>
<td>0.6914</td>
<td>0.5963</td>
</tr>
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<td>Resolved acrolein</td>
<td>0.9998</td>
<td>0.9526</td>
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<td>Mean</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>ML-sNMF</td>
<td>Resolved acetone</td>
<td>0.5495</td>
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<td>Resolved acrolein</td>
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<td>0.9526</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
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<td>NC</td>
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<td>Resolved acrolein</td>
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</tr>
<tr>
<td></td>
<td>Mean</td>
<td>NC</td>
<td>NA</td>
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NB: NC – Not Computed since the correlation co-efficient value is less than 0.9
NA- Not Applicable
Table 5.6  Signal recovery of resolved components using proposed ML-NMF algorithms and existing MCR-ALS algorithm on the separation of embedded chromatogram

<table>
<thead>
<tr>
<th>Algorithm category</th>
<th>Resolved components</th>
<th>Signal Recovery (dB)</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>Initialization category</td>
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<tr>
<td></td>
<td></td>
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<td>ML-NMFdiv</td>
<td>Resolved acetone</td>
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<td>Resolved acrolein</td>
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</table>

NB: NC – Not Computed since the correlation co-efficient value is less than 0.9
    NA – Not Applicable
5.4 SUMMARY

The proposed ML-NMF algorithms namely ML-NMFdiv, ML-sNMF and ML-NMFmse with three different initialization methods are used for the overlapped chromatogram separation and the results are compared with MCR-ALS method. The application of the above said algorithms on the simulated overlapped chromatograms of acetone and acrolein mixture show that the proposed method fails to perform better than MCR-ALS method for a partially (slightly) overlapped chromatogram; but performs better than MCR-ALS method for severely overlapped and embedded chromatograms. For the resolution of severely overlapped chromatogram, the ML-sNMF and ML-NMFmse algorithms along with EFA method of initialization outperforms all other methods as shown in Tables 5.3 and 5.4. In the case of embedded chromatogram, the ML-sNMF algorithms along with Robust method of initialization performs better than all other methods as shown in Tables 5.5 and 5.6. The ML-NMFmse algorithm is comparatively very simple due to less additional constraints imposed.

The results show that the proposed ML-NMF algorithms converge to a stable limit point and the resolution are found to be unique. The convergence and uniqueness are the advantages of the proposed ML-NMF algorithms. Hence, it can be concluded that the proposed ML-sNMF algorithm is a suitable choice for severely overlapped and embedded chromatogram separation.