An Algorithm for the Resolution of a Mixture of Preservatives with Overlapped Chromatogram

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Abstract—Resolution and quantitative determination of benzoic and sorbic acid preservatives in a mixture with overlapped High Performance Liquid Chromatography-Photo Diode Array detector (HPLC-PDA) Chromatogram was done using a newly developed algorithm. This algorithm had been tested both on a simulated data as well as on a real one-dimensional Chromatographic experimental data and the results were compared with the results of another Iterative Curve fitting algorithm developed and applied. The proposed method seems to be more efficient for both qualitative and quantitative analysis of a complex chemical mixture containing severely overlapping components.

Index Terms—Algorithm, overlapped Chromatogram, quantification, resolution.

I. INTRODUCTION

The use of permitted preservatives such as benzoic acid and sorbic acids must be carefully analyzed for their allowable concentrations in food stuff and beverages. Hence, the quantitative analysis of these products becomes essential for determining the quality in order to protect the paying public. The Association of Official Analytical Chemists (AOAC), 1990, approves the procedures 963.19 and 971.15 for the determination of benzoic and sorbic acids in food products. However these procedures counsel tiresome methods with broad extraction trial as well as involving huge amounts of reagents incurring significant amount of money. Reversed-phase HPLC [1–10], the most familiar analytical method for the determination of benzoic and sorbic acid or the parabens has been suggested, even though other systematic methods such as TLC [6], capillary electrophoresis [5,10] and gas Chromatography have also been reported. Most of the suggested procedures are for the separation of benzoic and sorbic acids. Simultaneous determination of benzoic and sorbic acid, using Chromatographic methods, especially in food items are scarce. This method has been gaining importance as there seems to be an increasing trend in using combination of preservatives, in pharmaceutical formulations and cosmetic products [7], along with the food industries.

Furthermore, many of the above mentioned methods involve tedious and intensive pre-treatments like steam distillation, multiple-steps and solid-phase extractions which requires several minutes [11] to separate and quantify the components. The Chromatographic method can also be suitably shortened using gradient elution technique which would replace the laborious separation procedures, reduce analysis time and expensive chemicals/equipments. They can be determined for quantification by techniques for resolving overlapped curves.

The recent literature shows lot of applications of the various resolution methods to Chromatographic analysis [12–14]. Cladera et al. utilized multiple linear regressions (MLR) in the resolution and quantification of binary and ternary mixtures of phenol compounds, which presented overlapped signals in HPLC. Hayashi et al. [15] proposed a one-dimensional Kalman filter to resolve partially overlapped Chromatographic peaks using a one-dimensional empirical model based on prior measurements of peak shape and location. Hayashi and Rutan [16] examined the accuracy and precision of the adaptive Kalman filter using computer simulations of Chromatographic situations, in which a known peak overlaps with an unknown (interferent) peak. A Kalman filter working on repetitive filtering of diode-array spectra obtained across a Chromatogram had also been developed [17]. Principal Component Regression (PCR) and Partial Least-squares Regression (PLS) were applied to multivariate analysis of overlapped peaks in gas Chromatography.

The entire above mentioned methods have a similar problem ie., when they are applied to resolve overlapping spectra, the degree of peak overlapping must be within a particular value. If the peaks are overlapping too strongly or completely, then the resolution results will not be acceptable. There had been many attempts and efforts to develop the resolution. Non-negative factor analysis was proposed by Paatero and Tapper to put an end to the resulting negative factors. When they performed factor analysis on procured information, they came up with alternating least squares (ALS) and positive matrix factorization (PMF) to solve the problem. Garrido Frenich et al. applied orthogonal projection approach (OPA), PMF and ALS to resolve multi-component peaks [18]. Hong-Tao Gao et al. applied non-negative matrix factorization for overlapped spectra resolution [19].

The present proposal utilizes different concentrations of benzoic and sorbic acid mixtures which were analyzed using HPLC-PDA for a flow rate of 1 mL.min⁻¹. Overlapping Chromatogram was obtained due to their similar retention time. Therefore, an algorithm has to be developed to resolve highly overlapped Chromatographic peaks. Further, it needs a calibration step by which the correlation between the chromatogram and the correspondent concentration can be inferred from a set of reference samples. The proposed algorithm resolves the overlapped Chromatogram in few seconds. The occurrence and quantification of benzoic acid and sorbic acid in edible products, for their Admissible Daily Intake (ADI) limit can be estimated in a shorter time than conventional experimental separation procedures which involve tough procedures, costly chemicals and equipments. The algorithm was also applied on another set of real

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experimental overlapped chromatogram obtained from Permethrin sample containing _trans_ and _cis_ Permethrin and it resolved the components perfectly. The results of the proposed algorithm were compared with the results of another Iterative Curve fitting algorithm developed and applied on the same experimental data and it shows that the former seems to be more efficient. The end result shows that the proposed algorithm could flawlessly resolve highly overlapping, smooth as well as non-smooth chromatograms in a short time and thus makes quantification easier.

II. MATERIALS AND METHODS

A. Experimental

Instrumental condition

A Jasco PU-1580 HPLC with photodiode-array detector was used. The chromatographic separations were performed using Qualisil BDS C18 column (250 x 4.6 mm I.D.). The detector was interfaced with an Intel Pentium 4 personal computer using Borwin software. The absorbance, wavelength, and time were digitized using the Borwin software, which allows representation and storage of data obtained at preset times.

Chemicals

Analytical standards of Sorbic and benzoic acid, solvent Acetonitrile and the other required pure water were obtained from a laboratory in Pondicherry University, Puducherry. Standard solutions of sorbic and benzoic acid were prepared by dissolving the appropriate amounts in Acetonitrile (ACN).

Procedure

A calibration matrix of sorbic and benzoic acid in the range 0.5mg of benzoic in 1mL of ACN and 0.2mg of Sorbic in 1mL of ACN were performed. Volumes of 20 mL were injected into the chromatographic system and the chromatographic separations were performed on a C18 column with a mobile phase of Acetonitrile; water (85:15 v/v) at a flow-rate of 1 mL min⁻¹ and a Wavelength of 254 nm. The solvent was filtered through Utipol N66 nylon 66 membrane filters, and degassed with Fast Clean Ultra Sonic Cleaner. Peak identification of these preservatives was based on the comparison with the retention times of standard compounds. For that purpose, standard solutions were prepared with Acetonitrile in the concentration of 0.5mg of benzoic acid in 1mL of ACN and 0.2mg of Sorbic acid in 1mL of ACN and chromatograms were obtained for the respective standards. Then the mixtures of sorbic and benzoic acid in different concentration (i.e., 0.5mg of benzoic acid in 1mL of ACN and, 0.2mg of sorbic acid in 1mL of ACN; 0.2mg of benzoic acid in 1mL of ACN and 0.5mg of sorbic acid in 1mL of ACN; 0.3mg of benzoic acid in 1mL of ACN and 0.2mg of sorbic acid in 1mL of ACN) were prepared and analyzed using HPLC-PDA at a wavelength of 254nm and with a carrier flow at the rate of 1 mL min⁻¹. The chromatograms obtained were resolved using the proposed algorithm and the results were compared with the results of another Iterative Curve fitting algorithm developed and applied on the same experimental data.

B. Algorithm

The chromatographic data (i.e., retention time _t_ and detector output _y_) have been exported to an ASCII file and then acquired through MATLAB R2008a software. An algorithm has been developed to resolve the overlapped chromatogram based on the crucial assumption that the chromatographic peak of a pure component is symmetric. The following steps have been followed in the proposed algorithm. Let the chromatographic data be _c(t,y)_

Step 1: Identification of first peak retention time _t_p1 at time _tn_ and detector output.

Step 2: 

\[
yl(j) = y(j) \\
tl(j) = t(j) \quad \text{for } j = 1 \text{ to } n
\]

Step 3: Identification of second peak retention time _t_p2 at time _tm_ and detector output _ym_.

\[
y2(j) = y(j) \\
t2(j) = t(j) \quad \text{for } j = m \text{ to end}
\]

Step 4: 

\[
y2(m-i) = y(m+i) \\
t2(m-i) = t(m-i) \quad \text{for } i = 1,2,3,\ldots(\text{end } - m)
\]

Step 5: The resolved Chromatogram of first component _C1_ = [ _tl, yl_ ]

Step 6: The resolved Chromatogram of second component _C2_ = [ _t2, y2_ ]

Step 7: Finding area of the resolved chromatograms and adjusting _C1_ and _C2_ until the sum of the areas of both resolved components matches with the area of overlapped chromatogram.

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![Fig.1 Resolved chromatogram obtained from proposed substitutive algorithm; Top figure denotes the simulated severely overlapped Chromatogram; the bottom figure denotes the resolved Chromatograms.](image-url)
RESULTS AND DISCUSSION

A. Results and discussion on simulated data

One dimensional data matrices of two-component system for partially and severely overlapped Chromatograms were obtained by cross product multiplication of two simulated Gaussian curves and tested using the algorithm developed.

The simulated Chromatograms and the resolved Chromatograms are shown in Figs. 1.

B. Results and discussion on experimental data (HPLC-PDA)

The analytical separation of sorbic and benzoic acid in the injected mixture using a Qualisil BDS C18 column (250 x 4.6 mm I.D.) was investigated. Acetonitrile-water were used as a mobile phases for performing the separation. The sorbic and benzoic acid were easily eluted by Acetonitrile-water and the retention time seemed to be closer. Fig.2 and Fig.3 shows the chromatograms for sample containing sorbic and benzoic acid in different concentration using Acetonitrile-water (85:15 v/v) as the mobile phase. Owing to the severely overlapping peaks, conventional measures of the different analytical signals (area or height of chromatographic peaks) cannot be realized.

Hence, the developed algorithm has been applied and tested on a real experimental one-dimensional chromatographic data of benzoic and sorbic acid mixture. The resolved chromatograms are given in Fig.2 and Fig.3 (middle and bottom figures).

C. Quantification and Calibration

After resolving the overlap, the areas of the individual components were determined. The calibration curve as given in Fig. 4 and Fig.5 has been drawn for quantification of individual components of a sample containing unknown concentration of benzoic acid and sorbic acid.

To compare the efficiency of the proposed algorithm, the same experimental data was resolved by another Iterative Curve fitting algorithm. But due to the non-smooth curve, the resolved result deviates more from the actual result. The results of the proposed algorithm and the Iterative Curve Fitting algorithm are compared in Table.1. It shows that the algorithm works well for a mixture of partially and severely overlapped components and it can also resolve non-smooth curves efficiently.

III. RESULTS AND DISCUSSION

A. Results and discussion on simulated data

One dimensional data matrices of two-component system for partially and severely overlapped Chromatograms were obtained by cross product multiplication of two simulated Gaussian curves and tested using the algorithm developed.

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chemistry. directly address very difficult problems in analytical chromatography, which shows prospect for the analysts to also enhance the qualitative identifying ability of the separation ability of a smooth and non-smooth curve but the proposed method in this paper can not only greatly enhance contains severely overlapping components. The above accurate analyses of real unknown complex systems that sample can be qualitatively and quantitatively analyzed.

From the above resolving process and the results obtained by using the algorithm developed upon the chromatographic data, one can see that the real complex systems that contains severely overlapping components. The above proposed method in this paper can not only greatly enhance the separation ability of a smooth and non-smooth curve but also enhance the qualitative identifying ability of the chromatography, which shows prospect for the analysts to directly address very difficult problems in analytical chemistry.

IV. CONCLUSION

Table 1a. Comparison of proposed algorithm with an Iterative Curve fitting algorithm on the resolving of overlapped chromatograms of 0.3 mg/mL of benzoic acid and 0.5 mg/mL of sorbic acid

<table>
<thead>
<tr>
<th>Resolved Component</th>
<th>Area of the resolved component using proposed algorithm</th>
<th>Error in Area of the resolved component using Iterative Curve fitting algorithm</th>
<th>Area of the resolved component using literature Curve fitting algorithm</th>
<th>Error in Area of the resolved component using Iterative Curve fitting algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid</td>
<td>8.553</td>
<td>+3.68%</td>
<td>8.02</td>
<td>+0.12%</td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>10.143</td>
<td>-3.5%</td>
<td>6.9412</td>
<td>-3.4%</td>
</tr>
</tbody>
</table>

Table 1b. Comparison of proposed algorithm with an Iterative Curve fitting algorithm on the resolving of overlapped chromatograms of 0.5 mg/mL of benzoic acid and 0.3 mg/mL of sorbic acid

<table>
<thead>
<tr>
<th>Resolved Component</th>
<th>Area of the resolved component using proposed algorithm</th>
<th>Error in Area of the resolved component using Iterative Curve fitting algorithm</th>
<th>Area of the resolved component using literature Curve fitting algorithm</th>
<th>Error in Area of the resolved component using Iterative Curve fitting algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid</td>
<td>9.537</td>
<td>-1.77%</td>
<td>9.7396</td>
<td>+3.39%</td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>6.105</td>
<td>-1.01%</td>
<td>6.75</td>
<td>-2.1%</td>
</tr>
</tbody>
</table>

REFERENCES