Reversed Phase-HPLC Method for Low Level Quantitation of Dimethyl dibenzylidene Sorbitol

Abdus Salam*
[1]*Department of Chemistry, Rabigh College of Science and Arts, King Abdulaziz University, Rabigh 21911, Post box 344, Saudi Arabia.*
asalam1@kau.edu.sa*

(Received on 26th April 2021, accepted in revised form 7th February 2022)

Summary: Dimethyl dibenzylidene sorbitol (DMDBS) is one of the most commonly used nucleating agent in polypropylene formulations. In present study, a simple and robust reversed phase liquid chromatography (RP-LC) based analytical method was developed for the quantitative low-level determination of DMDBS. The best separation was achieved on a RP-HPLC column TSK gel G2000 SW, 7.5mm x 30cm, Particle Size 10 µm from TOSOH Bioscience. A 0.05 M NH_4AC (pH = 6.6) + 10% ACN solution was used as mobile phase at a flow rate of 1.2 ml/min. UV detection was performed at 216 nm. Retention time was found to be 13.056 min. for DMDBS. The response was a linear function of concentration over the range of 2.00 to 8.00 µg/ml with correlation coefficient of 0.9996 for DMDBS. The LOD and LOQ for DMDBS were found to be 1.00 µg/mL and 2.00 µg/mL respectively. The method is simple, rapid and robust, which is suitable for application in quality control and can also be used for the estimation of DMDBS as a leachable/ extractable from polypropylene (PP) resin and resin products like medical device containers.

Keywords: Nucleating Agent, DMDBS, RP-HPLC, System Suitability, Linearity.

Introduction

Plastics are extensively being used in the manufacturing of various products including the packaging containers for medical devices, food and cosmetics etc. The plastic additives like plasticizers, lightstabilizers, antioxidants, and nuclear clarifying agents (NCAs)/ nucleating agents (NAs) are increasingly being used in polymers especially in polypropylene based polymers to improve their physical properties. Among the lot, three most commonly used sorbitol-based NAs available in the market with their brand names; Irgaclear, Irgaclear DM, and Millad have become a vital choice as polymer additives because of their improved physical properties, increased optical clarity, and facilitated nucleation during the production process [1].

Dimethyldibenzylidene Sorbitol DMDBS (Millad 3988); a nucleating agent/clarifier (Fig. 1), has become one of the most frequently used additives in the production of isotactic polypropylene (iPP). DMBDS together with iPP form a binary mixture with a monotectic phase diagram. At a reasonable temperature, a homogeneous dissolution of DMDBS in iPP is feasible only in low concentration ranges [2]. More specifically, a good clarification effect may be achieved by keeping DMDBS concentration below 1 w/w% in the mixture [3]. DMDBS addition makes polypropylene devices pleasingly attractive to the user and more cost effective when compared with other plastic products in the market [1]. When such plastics are used as containers for a pharmaceutical product, the additives like DMDBS, light-stabilizers, and antioxidants, are prone to leach out from plastics may cause contamination. In most of life science applications, the severity of substances leaching out of plastic devices is still not being addressed properly. Research studies showed that different undefined groups of chemicals may affect the experimental processes and impart errors in various assay systems [4]. Different scientific studies reveal that several NCAs were reported to interfere with biological assay systems and rated as potentially critical [5, 6].

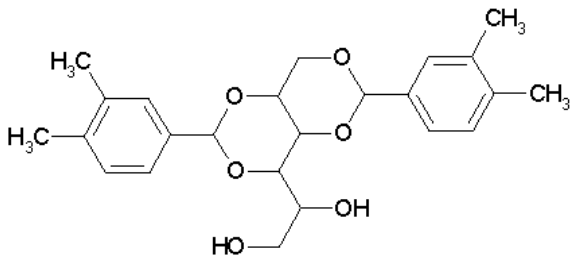

Fig. 1: Chemical structure of Dimethyl dibenzylidene sorbitol (DMDBS).

Although, a bulk of literature explaining the physical properties of NA's especially DMDBS are available [3, 7-13], limited literature is available on their analytical properties, such as mass spectroscopy and chromatography especially liquid chromatographic behavior for characterization and assay studies. Kim *et al*. had discussed his findings by analyzing the mass

*To whom all correspondence should be addressed.

spectrum of Irgaclear DM [14] while Feigenbaum *et al*. had conducted some extraction study of Irgaclear D from PP and analyzed the data using gas chromatography (GC) [15]. Jeffery *et al*. had extracted sorbitol-based NAs from general purpose polypropylene plasticware material and discussed their electron ionization mass spectra and collision-induced dissociation (CID) mass spectra [1].

As per recent literature survey, there is no simple and robust analytical method for the determination of DMDBS that has been reported. The proposed method is particularly useful in a fast working environment in industries like pharmaceutical, food and cosmetics industries. The current method is a straightforward and robust HPLC based technique for analytical characterization of DMDBS. The proposed method developed by utilizing an HPLC system equipped with an UV detector can also be used for the estimation of DMDBS as a leachable/ extractable from polypropylene resins and polypropylene resin products like medical devices and packaging materials.

Experimental

Chemicals and Reagents

All chemicals and reagents used were of the highest purity. Dimethyldibenzylidene Sorbitol (DMDBS) reference material available or high purity reagent (CAS #: 135861-56-2) acquired from Milliken Chemicals, with a manufacturer code name of Millad-3988. HPLC Grade Dimethyl Sulfoxide (DMSO), Methanol (MeOH), and Acetonitrile (ACN), were arranged from Fischer Scientific. Ammonium acetate (NH_4AC), AR Grade NaOH-1N and HCl-1N Solution AR Grade were obtained from BDH. Millipore Milli-Q system was used to obtain high-purity water.

HPLC Instrumentation

A HPLC system used for quantitative analysis consisted of a Waters Alliance System 2695 equipped with HPLC column heater module, an automatic injector with an injection volume of 20 µL and Waters 2487 dual wavelength UV absorbance detector. A RP HPLC column TSK gel G2000 SW, 7.5mm x 30cm, Particle Size 10 µm from TOSOH Bioscience was used. The control of HPLC system and data collection was done by Dell computer equipped with Empower 2 software. The column was maintained at 30ºC and eluted under isocratic conditions over 20 min at a flow rate of 1.20 mL/ min. A 0.05 M NH_4AC (pH = 6.6) + 10% ACN solution was used as mobile phase. UV detection was performed at 216 nm. HPLC system was wet primed for at least 15 minutes before performing any sequence and remove any air bubbles from HPLC system.

Preparation of Mobile Phase

To a 2000 mL Class A volumetric flask added about 7.708 ± 0.01 g of high purity grade NH_4AC. Diluted to the mark with high-purity water and vacuum filtered the solution through 0.45 µm Nylon filter membrane. Adjusted the pH = 6.6, if necessary, with 1N HCl. Added 200 mL of ACN, shake well and then degassed.

Preparation of Standard Solutions

Stock Standard Solutions

A stock standard solution was prepared by dissolving 100 mg of DMDBS into a 100 ml volumetric flask and diluted to the volume with DMSO (Concentration 1.0 mg/ml = 1000 ppm). Prepared in duplicate and labeled as Stock-1 and Stock-2.

Preparation of Intermediate Standard solutions

10.0 ml of each stock standard solution was taken into a separate 100 ml volumetric flask and made up to volume with MeOH (concentration 100 ppm). Two separate intermediate standards prepared from Stock-1 and Stock-2 were labeled as Intermediate Std-1 and Intermediate Std-2.

Preparation of Working Standards

5.0 mL of each Intermediate Standard Solution was taken into a separate 100 mL volumetric flask for working standard-1 and working standard-2 and made up the volume with MeOH (Concentration 5 ppm). Labeled as Working Standard-1 and Working Standard-2 (Verification Standard).

Preparation of Linearity Standard solutions

Linearity, Recovery, LOD and LOQ standard solutions were prepared from Intermediate Std-1 as mentioned in Table-1.

Table-1: Linearity standards over the range of 20 – 160% of the DMDBS nominal concentration.

Linearity Spiking Level (%LC)	DMDBS Concentration (ppm)	Dilutions
20%	1	1.0ml→100ml
40%	2	2.0ml→100ml
80%	4	4.0ml→100ml
100%*	5	5.0ml→100ml
120%	6	6.0ml→100ml
160%	8	8.0ml→100ml

*= Working Standard

Results and Discussion

For current studies, characteristics including System suitability, Linearity and Range (Correlation Coefficient), Precision (RSD, %), Accuracy (Recovery, % & RSD, %) and LOD & LOQ were investigated as per current ICH recommended method validation procedures [16]. All validation characteristics were evaluated against the limits given in United States Pharmacopeia (USP), which satisfy the criteria for linearity and accuracy, and yields an RSD of < 2% [17].

Linearity and Range

Within a specified range, linearity of an analytical method is its ability to produce test results that are directly proportional to the analyte concentration in a given sample [16]. The specified range is the interval between the upper and lower concentrations of the standard curve that meets the acceptance criteria of linearity, precision and accuracy. To evaluate the linearity and range of this method, calibration was carried out using a five-point standard calibration curve. The standard concentration ranging from 40-160% of the nominal concentration (5.00 ppm) at five different levels. The acceptance criteria of linearity data were judged through equation of straight line obtained from response versus concentration plot of different calibration standards. In general, the regression value (r^2-value) of ≥ 0.998 is considered as an evidence of acceptable fit of the data to the straight-line equation. Linearity and range data should also be used to calculate the percent relative standard deviation (%RSD), intercept, and slope of the regression equation.

In the current study, DMDBS concentration ranging from 2.0-8.0 µg/ml (40-160% of nominal concentration, n = 3) was used to obtained a regression equation by plotting the peak area response (y-axis) versus DMDBS concentration (x-axis) expressed in ppm (µg/ml): y = 36927x – 2311.9 (r^2 = 0.9996) as indicated in Fig. 2. Results as shown in Table-2, the calibration curve for dimethyldibenzylidene sorbitol exhibits excellent linearity and range with respect to peak area response vs. concentration, with correlation coefficients (r^2) of 0.9996 which is well within the acceptance criteria of r^2 ≥ 0.988. The percent standard deviation of the each five-point standard curve was less than 2%, which met the acceptance criteria of ≤ 2.0%. The range of a test method depends on its application and can be derived from linearity studies.

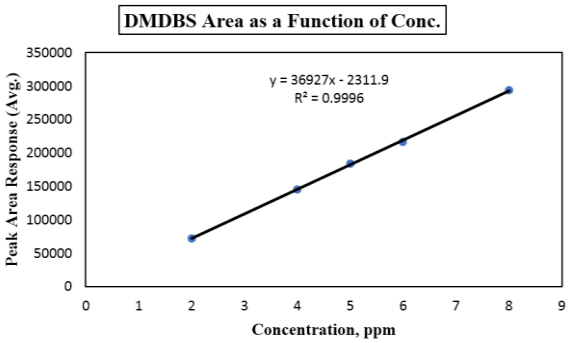

Fig. 2: Graph between the peak area and concentration for DMDBS demonstrating Linearity.

Table-2: Assessment results of Linearity of HPLC method for low level determination of Dimethyldibenzylidene Sorbitol (DMDBS):

Concentration (µg/mL, ppm)	Concentration as percent of 20 µg/mL	Peak Area Response as mean of 3 injections	Peak Area RSD (%)
2	40	71544	0.63
4	80	145272	0.50
5	100	184062	0.49
6	120	216566	0.76
8	160	293883	0.59

Correlation Coefficient (r^2) = 0.9996
Regression Line Equation: y = 36927x – 2311.9 (n = 3)
y-intercept = 1.26%

Method Accuracy/ Recovery Studies

The accuracy of an analytical method is the closeness of the data obtained by that method and the theoretical value. It is also a measure of recoveries when samples are prepared by spiking solvent/ solution with known amounts of analyte. Accuracy is demonstrated at three different levels over a range of 50-150% of the nominal concentration in triplicate (e.g., three concentrations, three replicates each). As there was no DMDBS sample available, the linearity data was used for accuracy and recovery. By following ICH2 guidelines, method accuracy was determined using recovery samples prepared at 80%, 100% and 120% of the nominal concentration of DMDBS (5.00 ppm). These samples were then quantified as percent recovery against bracketing standards. Percent recoveries were calculated from the response factor (area/concentration). Test results as shown in Table-3, the percent recovery for DMDBS for each level of concentration met the acceptance criteria of 100% ± 2%. In addition, the %RSD at each level was determined to be 1.0% and less with the acceptance criteria of ≤ 2.0%.

Table-3: Results of Recovery/ Accuracy of DMDBS from known concentration

Accuracy Spike level (%)	Concentration (ppm)	Recovery (%)	Avg. %Recovery (Limit 90-110%)	% RSD at each Level must ≤ 5%
80	3.9988	99.456 99.389 99.019	99.3	0.24
100	4.9985	100.868 101.326 101.862	101.3	0.49
120	5.9982	98.836 99.046 100.230	99.0	0.76

Mean Recovery: 99.9%; RSD: 1.25%

Limit of Detection and Limit of Quantitation

The procedure was tested for Limit of Detection (LOD) and Limit of Quantitation (LOQ) when the samples containing very low concentrations of analyte. LOD of any analytical procedure is the lowest detectable amount of an analyte which cannot be quantitated as consistent value; typically, three times the baseline noise level (S/N = 3). LOQ of an analytical method is the minimum possible amount of analyte present in a sample which can be determined quantitatively as an exact value that gives S/N = 10. In the current method, for a 20 µL injection of DMDBS standard, LOD was 1 ppm (1 µg/ml) with RSD < 5%, and the LOQ was 2 µg/ml with RSD < 1% (n = 3). As the DMDBS concentration is below 1 w/w% in various polymer mixture formulations, LOD and LOQ data reflect high sensitivity of the method which may be of key importance in most quantitative studies. Obtained results indicate that the developed method can also be used for detection and quantitation of analytes in low concentration range.

Specificity

Specificity of an analytical procedure is the ability to assess unequivocally the analyte in the presence of components like impurities, degradants, matrix, etc. which may be expected to be present [16]. In the current procedure, Fig. 3, provides a chromatogram for a reference standard of DMDBS which clearly demonstrates that DMDBS peak is well separated from any potential interference as no interfering peak was detected during the retention time (RT) of DMDBS peak. 20 µL of solvent (blank) for DMDBS was injected to investigate any possible assay interference. Fig. 4 clearly indicates the absence of any interfering peak. Therefore, the current method found specific for DMDBS.

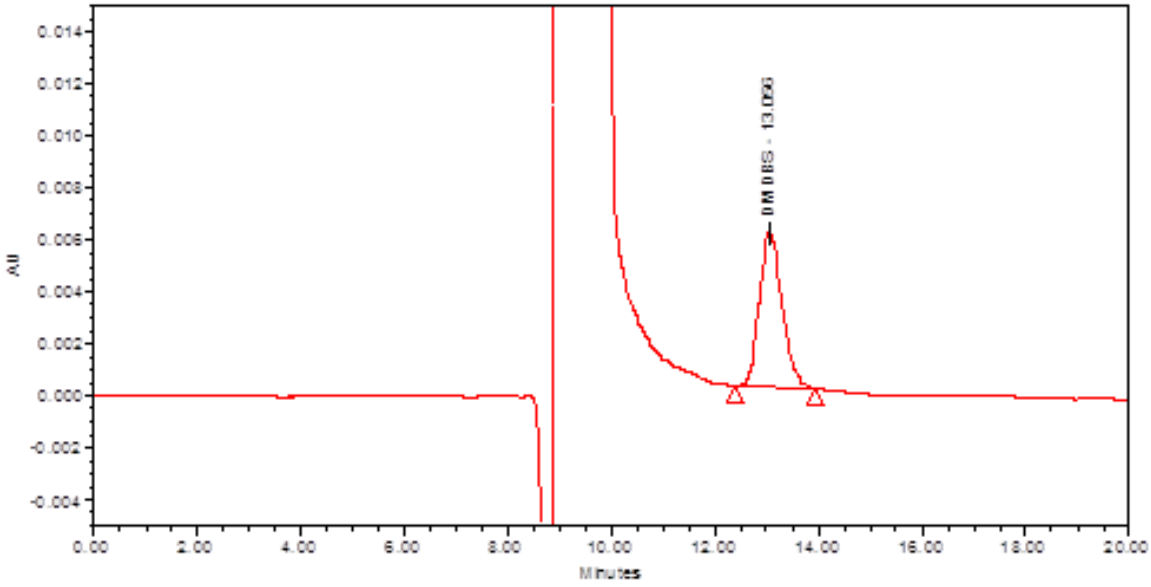

Fig. 3: Typical HPLC Chromatogram of Working Standard DMDBS.

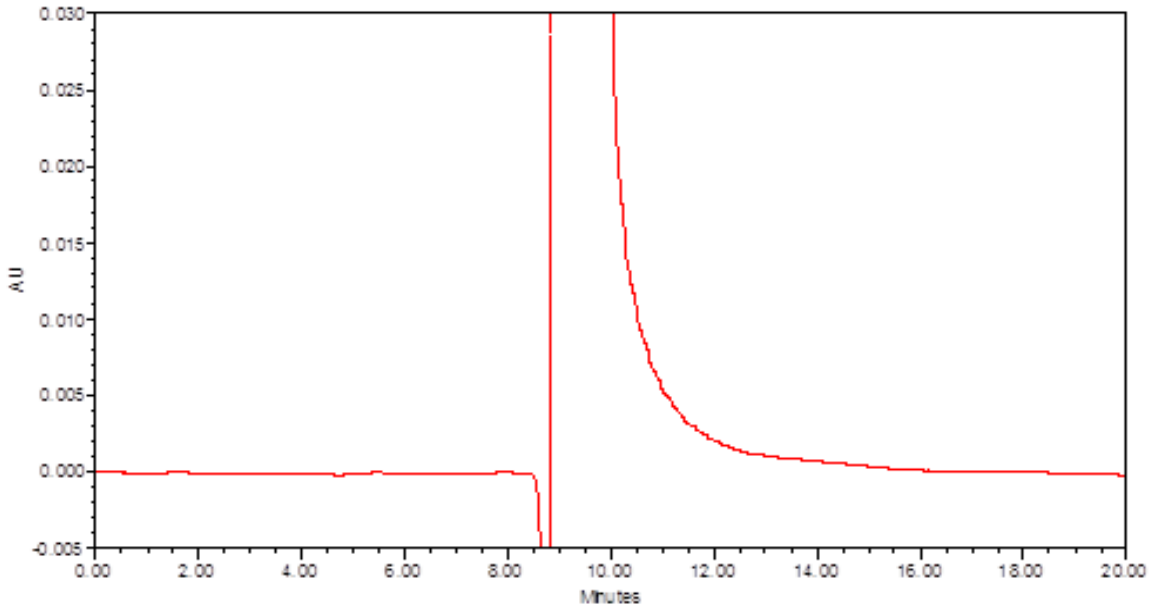

Fig. 4: HPLC Chromatogram of solvent (Blank).

Table-4: Summary of System Suitability (System Precision) for determination of DMDBS.

System Suitability Parameters	Acceptance Criteria	Results
Injections precision for peak area of working standard (n = 6)	RSD ≤ 2%	(1%) 0.88%
Standard agreement	≤ ± 2% (98 - 102%)	99.8%
Tailing Factor (T)	T ≤ 2	1.19
Theoretical Plates (N)	N ≥ 2000	4200
Blank	No interference at RT of DMDBS peak	Conforms
% System Drift (n=10)	RSD ≤ 2%	(1%) 0.91%

System Suitability

System suitability test ensures the validity of a method and an analytical system before accepting the data. The parameters, like retention time, pressure, resolution, repeatability, column efficiency, plate number, tailing factor and signal-to-noise ratio are determined during the system suitability testing and compared the respective results against the specifications set for the method in question [16]. The parameter to be measured and their recommended limits [16, 17] obtained from system suitability test analysis of a sample are shown in Table-4. In the present study, the system suitability test was performed on the HPLC system to determine the accuracy and precision of the system, by injecting six injections of a working standard solution containing 5 µg/ml of DMDBS. RSD for peak area < 1%, tailing factor (T) < 2 and theoretical plate (N) were > 4000 for the HPLC system. The system drift that is the RSD of all the working standards injected during a complete run (n=10) was < 1% as can be seen in Table-4.

Conclusion

In this study, a novel, reliable and robust RP-LC based analytical method was developed for the quantitative low-level determination of dimethyl dibenzylidene sorbitol (DMDBS). Key advantages of this method are its easy-to-use and simplicity. This method was developed by utilizing an HPLC system equipped with an UV detector which is the most common and accessible combination in chromatography. This method enables simple, selective, sensitive and specific analysis of DMDBS purity. The current method can equally be useful for the quantitative determination of DMDBS as leachable/ extractable in polypropylene resins and resin products like medical and food package devices in aqueous and non-aqueous mediums.

Acknowledgement

This work was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under the grant No. D1437-23-662. The author, therefore, acknowledges with thanks DSR technical and scientific support.

References

1. J. G. McDonald, C. L. Cummins, R. M. Barkley, B. M. Thompson and H. A. Lincoln, Identification and Quantitation of Sorbitol-Based Nuclear Clarifying Agents Extracted from Common Laboratory and Consumer Plasticware Made of Polypropylene, *Anal. Chem.*, **80**, 5532 (2008).
2. L. Balzano, S. Rastogi and G. Peters, Nano-structuring of isotactic polypropylene in presence of a self-assembling nucleating agent under defined flow conditions, *NSTI-Nanotech*, **2,** 820 (2006).
3. L. Balzano, G. Portale, G. W. M. Peters and S. Rastogi, Thermoreversible DMDBS Phase Separation in IPP: The Effects of Flow on the Morphology, *Macromolecules*, **41**, 5350 (2008).
4. G. R. McDonald, A. L. Hudson, S. M. Dunn, H. You, G. B. Baker, R. M. Whittal, J. W. Martin, A. Jha, D. E. Edmondson and A. Holt, Bioactive contaminants leach from disposable laboratory plasticware, *Science*, **322**, 5903 (2008).
5. T. W. Lee, S. Tumanov, S. G. Villas-Bôas, J. M. Montgomery and N. P. Birch, Chemicals eluting from disposable plastic syringes and syringe filters alter neurite growth, axogenesis and the microtubule cytoskeleton in cultured hippocampal neurons, *J. Neurochem.*, **133**, 53 (2015).
6. M. Hammond, L. Marghitoiu, H. Lee, L. Perez, G. Rogers, Y. Nashed-Samuel, H. Nunn and S. Kline, A cytotoxic leachable compound from single-use bioprocess equipment that causes poor cell growth performance, *Biotechnol. Prog.*, **30**, 332 (2014).
7. Y. Zhang, X. Li and X. Wei, Non-Isothermal Crystallization Kinetics of Isotactic Polypropylene Nucleated with 1,3:2,4-Bis(3, 4-Dimethylbenzylidene) Sorbitol, *J. Therm. Anal. Calorim.*, **100**, 661 (2010).
8. A. S. Vaughan and I. L. Hosier, The Effect of Dibenzylidene Sorbitol on the Crystallization Behaviour of Polyethylene. *J. Mater. Sci.*, **43**, 2922 (2008).
9. S. Liu, W. Yu and C. Zhou, Molecular Self-Assembly Assisted Liquid-Liquid Phase Separation in Ultrahigh Molecular Weight Polyethylene/ LiquidParaffin/ Dibenzylidene Sorbitol Ternary Blends, *Macromolecules*, **46**, 6309 (2013).
10. K. Sreenivas, H. V. Pol and G. Kumaraswamy, The Influence of DMDBS on the Morphology and Mechanical Properties of Polypropylene Cast Films, *Polym. Eng. Sci.*, **51**, 2013 (2011).
11. P. Supaphol, P. Charoenphol and J. Junkasem, Effect of Nucleating Agents on Crystallization and Melting Behavior and Mechanical Properties of Nucleated Syndiotactic Poly(Propylene), *Macromol. Matr. Eng*, **289**, 818 (2004).
12. W. Lai and L. Cheng, Preparation and Characterization of Novel Poly (Vinylidene fluoride) Membranes Using Self-Assembled Dibenzylidene Sorbitol for Membrane Distillation, *Desalination*, **332**, 7 (2014).
13. Y. Zhao, C. Yao, T. Chang and Y. Zhu, The Influence of DMDBS on Crystallization Behavior and Crystalline Morphology of Weakly-Phase-Separated Olefin Block Copolymer, *Polymers*, **11**, 552 (2019).
14. S. H. Kim, C. M. Shin and J. S. Yoo, First Application of Thermal Vapor Deposition Method to Matrix-assisted Laser Desorption Ionization Mass Spectrometry: Determination of Molecular Mass of bis(p-methyl benzylidene) Sorbitol, *Rapid Commun. Mass Spectrom.*, **12**, 701 (1998).
15. A. Feigenbaum, D. Scholler, J. Bouquant, G. Brigot, D. Ferrier, R. Franz, L. Lillemark, A. M. Riquet, J. H. Petersen, B. Van Lierop and N. Yagoubi, Safety and Quality of Food Contact Materials. Part 1: Evaluation of Analytical Strategies to Introduce Migration Testing into Good Manufacturing Practice, *Food Addit. Contam.*, **19**, 184 (2002).
16. Validation of Analytical Procedures: Text and Methodology, *ICH, Q2(R1), FDA.*, (2005).
17. Chromatography, USP 41-NF 36 (621), *United States Pharmacopeal Convention*, Rockville, MD, **4**, 6363 (2018).