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Original Article

APPLICATION OF MULTIVARIATE CURVE RESOLUTION ALTERNATING LEAST SQUARE (MCR-ALS) TO THE STUDY OF TRIKARSHIKA FORMULATION

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ABSTRACT

Objective: Multivariate curve resolution method (MCR) is one of the tools designated to unravel the pure component mathematically. The information was extracted through alternative least square algorithm from Trikarshika (TK) formulation. This paper presents the importance of MCR technique in the computation of mathematical component in the form of extract (TKChurna) itself.

Methods: The methanolic macerated extracts of the drugs i.e. *Zingiber officinale* Rose., *Aconitum heterophyllum* Wall. and *Cyperus rotundas* Linn, processed with standard operating procedure and prepared twenty-five mixtures to blend in three concentration levels among the individual simultaneously. The finished mixture annotated with code numbers and considered as an individual sample. The samples were subjected to ultra violet –visible (UV-VIS) UV-VIS spectral detection from 220 to 780 nm at the interval of 10 nm. The data were analyses with the help of The Unscrambler software.

Results: Seven components were computed from TK mixture extracts using concentration as a factor. The correlation between estimated and reference concentration for the *Zingiber officinale* Rose. was 0.91, this indicates good predictability of *Zingiber officinale* Rose in comparison to other two ingredients. Several spectral overlap existed only generated the use of absorbance resolved spectra that open up the scope of mixture analysis based on experimental data only.

Conclusion: MCR technique may be a good tool that can be adopted for the discrimination of variables like exposes of discrete light, seasonal differences in the collection, and differences in harvesting schedules. The discrimination of MCR technique is based on ALS algorithm.

Keywords: Trikarshika, Multivariate curve resolution, UV-Vis spectroscopy, Unscrambler Camo Software

INTRODUCTION

Indian system of medicine is now in a commendable position due to a rich source of natural flora. But existing status is more focused to globalize Ayurveda with the help of reverse, system and ethnopharmacology concept [1]. Most of the classical Ayurvedic formulation is the comprised of not less than ten ingredients that the assessing the quality and quantity of each ingredient is very difficult due to the complex nature of the herbal ingredients. In another context, the whole drugs were used by Ayurvedists of the olden times for a reason not only the pharmacological and economic but also social i.e. relating to the tastes, habits, customs and social conditions. Ayurvedist, on the other hand, has attached more importance to the clinical findings and have based the pharmacological value of the whole drugs. The principle drug act as a whole there is no need for isolation of primary and secondary plant metabolites due to belief in the holistic sense. They have again used extracts (Sattva, Kwath and etc.) occasionally, evidently for the purpose of portability and adaptability and also for the facility of concentration. Accepting the extraction technique individual extract take as a marker then prepared artificially blended mixture as a concentration factor. We aim to understand quality in terms of the agreement between an expected or declared a property with the results of a suitable measurement of that property with helping of higher order chemometrics technique i.e. MCR. It can be considered as a ''sleeping giant of chemometrics'' with slow but persistent growth [2]. Various biochemical process precisely studied by spectroscopic monitoring, and multivariate resolution techniques include protonation and complexation of nucleic acids and other numerous events such as drug interaction processes and salt, solvent, or temperature–induced conformational transition. [3-8]. Photodegradation study of melatonin successively explained with the help of multivariate curve resolution in UV-VIS spectroscopy. [9]

Any Multicomponent system is basically transforming the raw experimental measurement into useful information. To obtain a clear description of the contribution of each of the components present in the mixture. It is the process from the overall measured variation in the chemical data. Ayurvedic pharmacopeia established their protocol as a single or finished product in favor of pharmacognosy and physicochemical parameter, thin layer chromatography fingerprinting. The risk of distortion of Ayurvedic concept and approaches is challenged to establish stable elementary quality control department due to the reprehensive profile of authenticated sample is not fully available.

But recent years that quality judgment by determining selecting one or two markers ignoring the complexity of the herbal medications is no longer the keynote of people's thinking pattern, and have acquired consensus that the comprehensive strategy for assessing the intact quality of herbal medicine is necessary [10]. Ancient Ayurvedic lexicon [11] highlights a polyherbal formulation (TK) for appetite enhancing, digestive, febrifuge actions. Now a day's various well-developed instruments are facilitated to obtain large amounts of data in a quick time. In the light of the above background, the present study was designed and undertaken to study of TK formulation in favour of non–destructive method (UV-VIS spectroscopy) along with very flexible multivariate way data analysis (MCR) method based on the assumption of Lambert-Beer's Law. MCR methods defined as a technique which intends the recovery of concentration (chemical composition changes) and response profile (spectra) of the components in an unresolved mixture using a minimal number of assumptions about the nature and composition of these mixtures. This method is excellent for the predictable tool only overlapping spectra of relative concentration. This type of method is provided an extensive diagnostic tool that gives an indication of the quality of prediction and also can be adopted for

the discrimination variables like exposes of discrete light, seasonal differences in collection and differing in harvesting season.

MATERIALS AND METHODS

Chemical and Experimental Laboratory spectroscopic grade methanol was purchased from Merck, India. Spectrophotometric measurements were recorded using a double-beam Systonic spectrometer (2201). Chemometric tool MCR-ALS algorithm was performed by a free trial version of "The Unscrambler®9.7 (CAMO Software AS).

Sample preparation

Sunthi, ativisha and musta samples collected from the Ayurved University of Gujarat, Department of Rasasastra and Bhaisajya-Kalpana and authenticated by the Pharmacognosy department of Gujarat Ayurved University as voucher no-GAU/6020/2012. The individual drug extracted in methanol by the maceration process. Then after drying in water bath again dissolved in methanol, applying sonication just for 20 minutes again drying in water-bath**,** redissolved the solvent at the solvent methanol and prepared stock solution 1 mg/ml of individual drug. Afterward the twenty-five mixture blends prepared at the known concentration in the combination of the respective individual extract (Table-1). The respective samples were subjected to UV-VIS spectral detection from 220-780 nm at the interval of 10 nm before doing cell matching using a blank (methanol).

Multivariate curve resolution (MCR)

Multivariate Curve Resolution is defined as a well-known chemometric technique which helps un-resolved mixtures by determining the number of components with their response profiles (spectra) and their concentrations when no prior information is available about the nature and composition of these mixtures. It is a well-established technique for evaluating and soft-modeling evolving systems and two-dimensional correlations spectroscopy (2DCoS), a method that is spreading the modulated spectra over a second spectral dimension, thus enhancing spectral resolution [12]. A very flexible two-way data analysis method based on the assumption of Lambert-Beer's Law, MCR decomposes the experimental data matrix D into the product of two smaller matrices C and S^T, with C being a matrix of concentration profiles for each modeled component in the system and S^T being the matrix of the corresponding pure spectra. E is the error matrix, i.e., the residual variation of the data set that is not related to any chemical contribution.

$$D = CS^T + E \quad \ldots\ldots (1)$$

The number of components (chemical species) contributing to D and to be modeled by MCR has to be determined, and initial estimates for C or S^T have to be provided. Then C and S^T are optimized iteratively in an alternating least squares (ALS) algorithm until convergence is reached. At each cycle, a new estimation of S^T and C is calculated by solving the following least square matrix equation alternatively.

$$S^T = (C)^+D \quad \ldots\ldots (2)$$

$$C = D\,(S^T)^+ \quad \ldots\ldots (3)$$

Where $(S^T)^+$and $(C)+$are the pseudo inverse of the S^T and C matrices, respectively. During the optimization step, another constraint can be applied to drive the final solution towards a chemical meaning. Non-negativity constraint forces the concentrations and the spectra of the components so that must be positive; the concentration profiles in the Chromophore interaction process are forced to give only one maximum per experiment by unimodality constraint [13]. Applied constraint (mathematical and chemical property systemically) fulfilled by the whole system or by some of its pure contributions. Multivariate Curve Resolution is an extensive diagnostic tool that gives the researcher an indication of the quality of the prediction, so user are not left in the dark.

Data analysis

Data is utilized UV-Vis spectra of TK blend to extract pure sample spectra and their relative concentration. The samples consist of 25 spectra of mixture samples. However, probes 1 to 3 were semi–purified extract of sunthi, ativisha and musta [Table-1]. On the contrary the variables, first three variables are concentration measurements. Variables 4 to 60 are UV-VIS absorbance measured at range 220-780 nm with the interval 10 nm. The data were exported in the form of single profiles by both concentration and absorbance as a matrix form. Afterward the experimental data executed by the MCR algorithm on Unscrambler software.

Table 1: Calibration set of TK formulations

S. No.	Sunthi	(µg/ml)	Ativisha (µg/ml)	Musta (µg/ml)
1	Probe	-1 3	0	0
2	Probe	-2 0	3	0
3	Probe	-3 0	0	3
4	Mix	-1 1.5	0.5	1
5	Mix	- 2 1.5	1	0.5
6	Mix	-3 0.5	1	1.5
7	Mix	-4 0.5	1.5	1
8	Mix	-5 1	0.5	1.5
9	Mix	-6 1	1.5	0.5
10	Mix	-7 1.5	0.5	1
11	Mix	-8 1.5	1	0.5
12	Mix	-9 0.5	1	1.5
13	Mix	-10 0.5	1.5	1
14	Mix	-11 1	0.5	1.5
15	Mix	-12 1	1.5	0.5
16	Mix	-13 1.5	0.5	1
17	Mix	-14 1.5	1	0.5
18	Mix	-15 0.5	1	1.5
19	Mix	-16 0.5	1.5	1
20	Mix	-17 1	0.5	1.5
21	Mix	-18 1	1.5	0.5
22	Mix	-19 1.5	0.5	1
23	Mix	-20 1.5	1	0.5
24	Mix	-21 0.5	1	1.5
25	Mix	-22 0.5	1.5	1
26	Mix	-23 1	0.5	1.5
27	Mix	-24 1	1.5	0.5
28	Mix	-25 1	1	1

RESULTS AND DISCUSSION

In this study, evolutionary process, as MCR algorithm applied in favor of strongly overlapped absorption bands of the different components on the contrast of (TK) mixer blend. MCR algorithm is explained on pure variable selection from PCA loading to find an initial estimate of spectral profile. ALS algorithm resolved the optimized spectra and concentration profile. Usually here an assuming minimum information of the mixture blend which had responded as absorbing species (Chromophore, conjugation, etc) in the same spectral region. In those circumstances mixer concentration profiles assumed the UV-VIS Chromophore sensitive compound (table 2) indirectly responsible for the absorbance of various wavelengths.

Table 2: Complete list of UV-Vis sensitive compound alignment of registered wavelength [14]

Chromophore	Structure	λ (nm)	Mol. Abs (l/mol/cm)
Aldehyde	-CHO	280-300	11-18
Amine	-NH2	145	2800
Bromide	-Br	208	300
Carbonyl	-C=O	195,270-285	1000, 18-30
Disulfide	-S-S	194, 255	5500,400
Ester	-COOR	205	50
Ether	-O-	185	1000
Nitrile	-ONO	220-300	5000
Oxime	-NOH	190	4000, 1600
Thio ether	-S	194	8000
Alkyne	-C=C,$(C=C)_2$,$(C=C)_3$	190,210-230, 260	8000,21000,35000
Carbonyl	-C=C-C=O	210-250	10000-20000
Benzene	Aromatic ring	184,204,255	46700, 6900,170
Diphenyl	Link Aromatic	246	20,000
Anthracene	Fused aromatic ring	252	199,000

Assess the total number of pure component

Mathematically assessing the number of components, we utilized UV-VIS spectra of the blend to extract pure component spectra and their relative concentration. The estimated seven pure spectra (fig. 1.-A) of taking whole wavelength (220-780 nm) absorption value. On the estimated concentration in favor of sunthi, ativisha and musta mathematically seven components (fig. 1.-B) profile across all calibration samples mixture by applying MCR technique.

In the context of UV-VIS spectroscopy for various Chromophore sensitive (aldehyde, amide, carbonyl, biphenyl, etc.) are adequated to for data variation in the chemical sense along with concentration as a factor. Because in extract level (TK) phytochemical screening was reported flavonoids, steroids, tannins, carbohydrate and protein [15]. On the other sense flavonoids, anthracene, phenol aglycones, the presence of various functional contributing groups in the structure might be the responsible of solubility of respective TK methanolic extract.

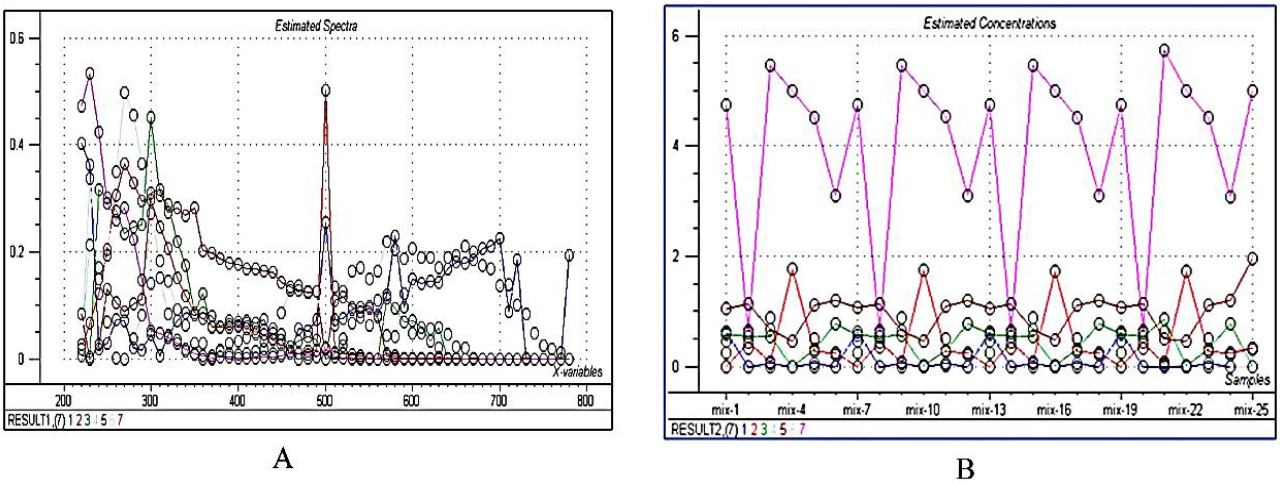

Fig. 1: (A) Estimated spectra reflect their data point of in abscissa (220-780 nm wavelength) and ordinate (absorbance) and (B) shown estimated concentration profile optimum seven number component of applying MCR selected mixture

Assess the pure sensitive component

In MCR, TK mixture resolved into seven pure components. The number of components and their concentrations and instrumental profiles estimated in a way that explained the structure of the observed data under the model constraint. Here identified the components 2, 3, 4, 5, 6 and 7 and their respective residual using estimated spectra (200-780 nm) [fig. 2]; After assessed various iteration, it shows that four-five components spectral pattern are same. [fig. 2: (D),(E)] because the residual is not more changed. At the chemical point of view, Trikarshika blend empirically gave positive correlation with pure Gallic acid (Total Phenol), quercetin (Total Flavonoid) [15]. Three estimated component has seen less overlap among each other. On the supplementary, when selecting the wavelength (300-370 nm) as a variable, their only get three pure components and their resolution here not shown.

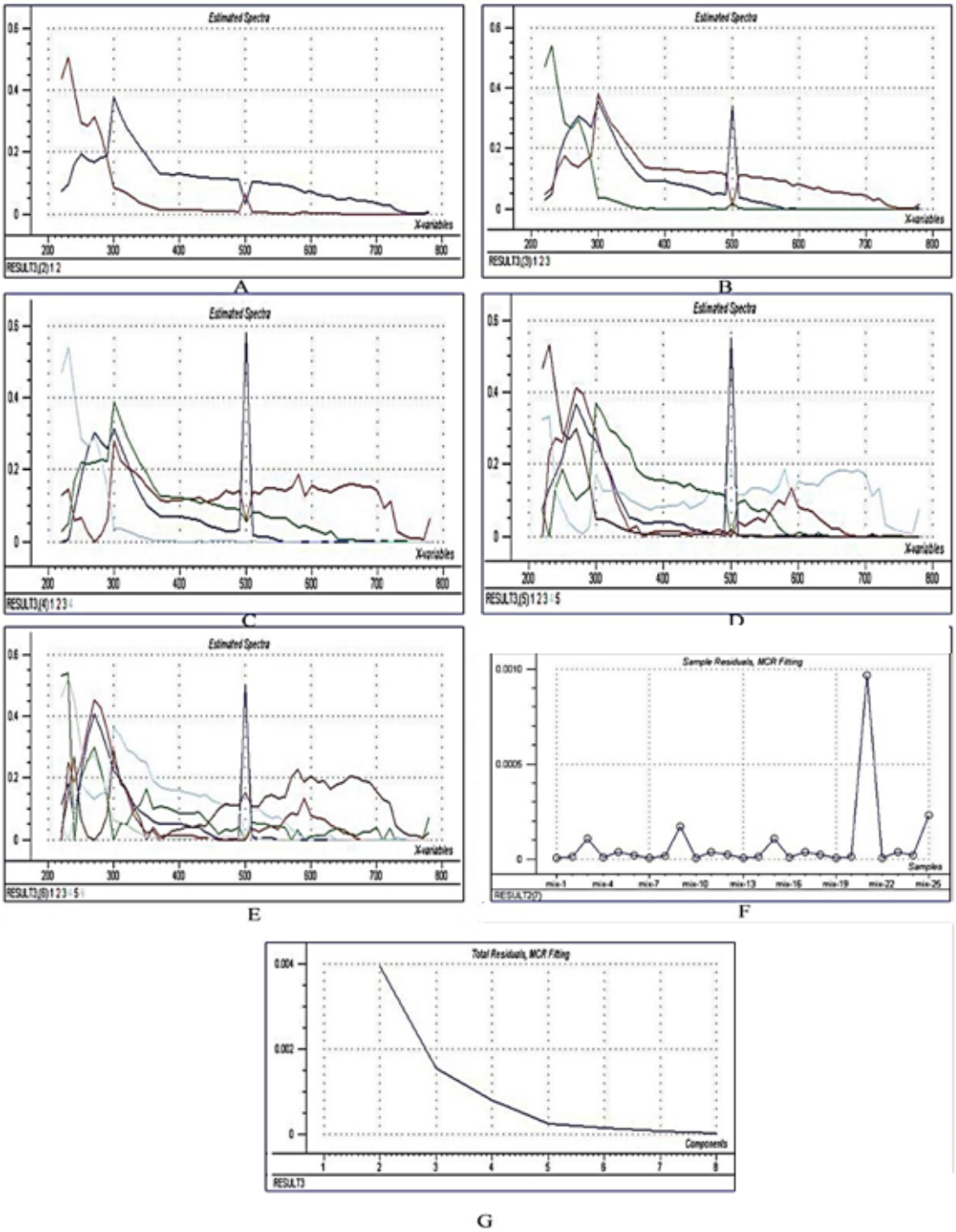

Fig. 2: Increasing the no. of pure estimated spectral components 2 (A), 3 (B), 4 (C), 5 (D), 6 (E) and 7 (F), their sample residual (F) and total residual (G) in MCR model

Compare the estimated concentration and spectra to the reference concentration and spectra

In the Validation norm, the estimated result (concentration) by initial guess MCR with a reference line spot of true table (concentration profile) is shown graphically intuition for more or less common pattern in musta sample both (fig 3 (A), (B)).

The estimated spectral profiles compared to the reference spectral profiles in the same way as for the concentrations. Because we used the spectra as initial guess inputs in this example, the comparison shows a perfect match. However, estimated spectra are an unit vector normalized; they are not the "real" spectral profile of the samples. Here 2nd estimated spectral profile similar to the reference profile of the red *(*Ativisha*).* (fig 3 (C), (D)).

MCR results in matrix form

The correlation between estimated and reference concentration for the *sunthi* 0.913412 [Fig-4] these very high correlations indicated that the MCR calculations have determined concentration profiles accurately in this case. In the same way ativisha musta have is negatively correlated. Here activist *and* musta correlation curve here not given.

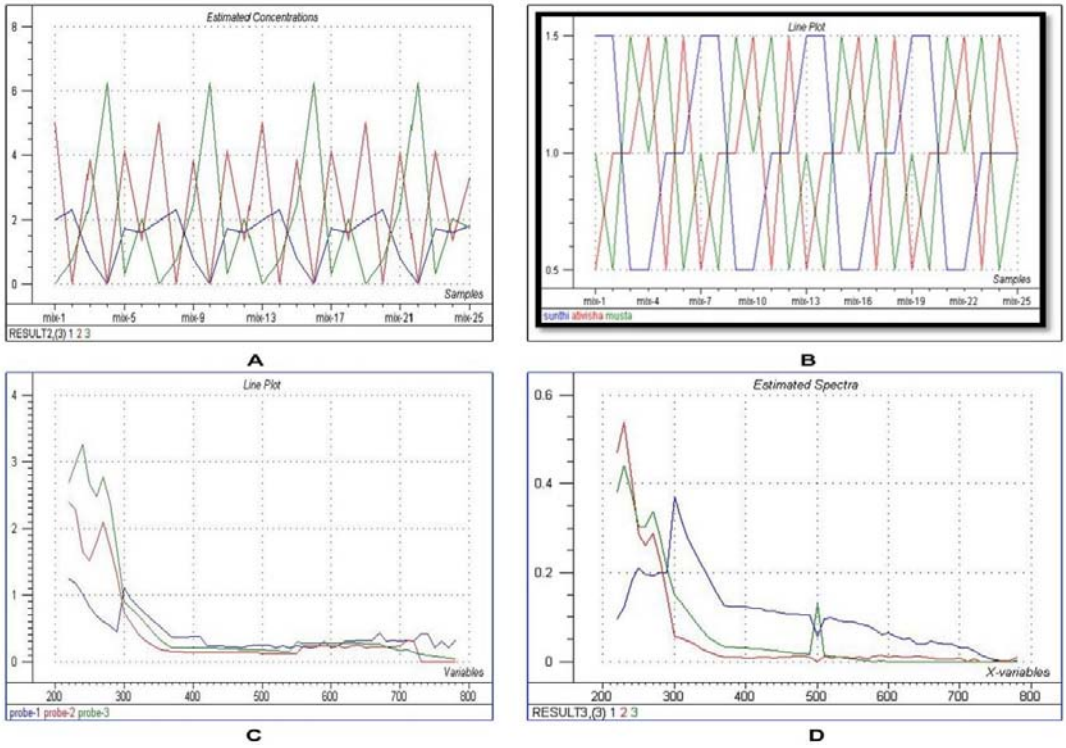

Fig. 3: Compare the estimated concentration and spectra to the reference line spot of concentration and spectra

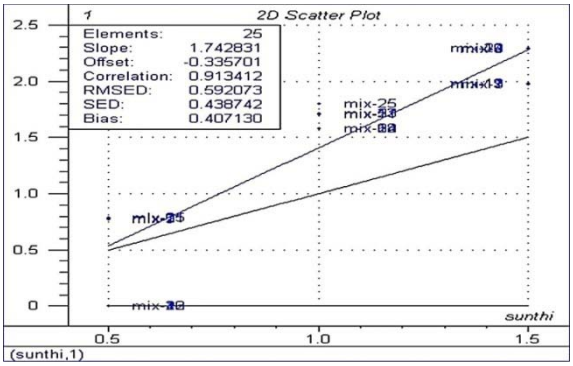

Fig. 4: Scatter plot of original and estimated sunthi concentrations from the MCR model

CONCLUSION

The Ayurvedic drug acts as a whole, no need of isolation any plant secondary metabolites (other active ingredients) which present with other phytoconstituents. This often called matrix effect. Some assumption also overwhelms drug act as a whole, holistic approach. In quality control of any particular formulation, one and two markers approach not a sound tone to evaluate that particular formulation. Today's era is full of technology, Automation that reflects easily whole chemical properties in a time of fraction. Crude natural product generally extracts an extremely complicated mixture of several compounds possessing varying chemical and physical properties. Alcoholic extract–plant materials contains a wide variety of polar and moderately polar (glycosides, steroids, flavonoid, etc) compound, by virtue of the co-solubility. UV-Vis spectroscopy participated only chromophore in giving distinct absorption profile. The research field of quality control of polyherbal formulation is really interdisciplinary approach. Chemometrics technique (MCR, etc.) is to provide a platform for the quality control of traditional medicines and further to discover the novel approach derived mathematical component. In this study, an application of multivariate curves resolution to resolve mixture profile in a graphical way also validated in both concentrations and spectra. Sunthi concentration shows high linear correlation (0.9134) in the respective mixture profile. In that way, you can solve various overlapping peak related problem. Also, evaluate the polyherbal formulation to store document as a spectral library.

CONFLICT OF INTERESTS

Declared none

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