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Spectrophotometric multicomponent determination of quercetin, berberine hcl and curcumin by double divisor ratio spectra derivative method

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Abstract
Double divisor-ratio spectra derivative method based on the spectrophotometric data was developed for the simultaneous analysis of a ternary mixture containing quercetin, berberine HCl and curcumin in phosphate buffer pH-7.4. This method is based on the use of the derivative of the ratio spectrum obtained by dividing the absorption spectrum of the ternary mixture by a standard spectrum of a mixture of two of the three compounds in the title mixture. The concentrations of three phytoconstituents in their mixture were determined by using their respective calibration graphs which are obtained by measuring the amplitude at either the maximum or minimum wavelengths selected. The selected wavelengths for determination of quercetin, berberine HCl and curcumin are 355.11 nm, 264.68 nm, and 399.15 nm, respectively. The mean % recoveries were found to be in the range of 98.66 - 99.23%, 99.67 - 100.56 % and 98.28 - 99.54 % for quercetin, berberine HCl and curcumin, respectively. The method was successfully applied to analyze quercetin, berberine HCl and curcumin in polyherbal gel formulation with no interference from excipients as indicated by the recovery study. All validation parameters were well within the acceptable range.

Keywords: quercetin, berberine HCL, curcumin, double divisor ratio spectra derivative spectrophotometry, simultaneous analysis

Introduction

Berberine (5, 6-dihydro-9, 10-dimethoxybenzo[g]-1, 3-benzodioxolo [5, 6-a] quinolizinium) is a nonbasic and quaternary benzylisoquinoline alkaloid and is bright yellow color. Berberine is present in plants such as barberry(*Berberis vulgaris*), tree turmeric (*Berberis aristata*), Oregon grape *(Mahonia aquifolium*), golden seal *(Hydrastis canadensis*), Chinese gold thread (*Coptis chinensis*), yellow root *(Xanthorhiza simplicissima*), Amur cork tree *(Phellodendron amurense*), prickly poppy *(Argemone mexicana*) and Californian poppy *(Eschscholzia californica*). It possesses number of activities such as antiacne, antimicrobial, antioxidant, antitumor, anti-inflammatory, and anti-diabetic, wound healing [1-3].

Quercetin (QCN) is a flavonol, which is a subclass of flavonoids and is present in number of fruits and vegetables such as onion (*Allium cepa* Linn.), apple (*Malus pumila* Miller), Black tea (*Camellia sinensis* (L.) Kuntze) and black currant (*Ribes nigrum* Linn). It possesses number of activities such as antiacne, antimicrobial, antioxidant, anti-carcinogenic, anti-inflammatory [4-6].

Curcumin is yellow in color, chemically known as diferuloylmethane and derived from the plant *Curcuma longa* Linn and. It possesses number of activities such as antiacne, antioxidant, anti-inflammatory, antiviral, antibacterial, antimicrobial, antifungal and anticancer. Curcumin is used in the treatment of arthritis, Alzheimer's disease, diabetes and skin disease [7-9].

Estimation of berberine HCl has been done by HPLC, HPTLC and UV technique individually and also in combination with other compounds [10-16]. Estimation of quercetin has been done by using HPLC, HPTLC and UV technique individually and also in combination with other compounds [17-21]. Estimation of curcumin has been done by using HPLC, HPTLC and UV technique individually and also in combination with other compounds [10, 16, 21-26].

Even though various analytical methods are available in literature for quantification of quercetin, berberine HCl and curcumin individually and also in combination with other compounds, no method is available for their simultaneous determination. Since quercetin, berberine HCl and curcuminoids have strong absorption in UV light, it was thought worthwhile to develop a spectrophotometric method for the simultaneous determination of these phytoconstituents.

Material and Methods

Reagents and Chemicals

All material and reagents were analytical grade. Quercetin, berberine HCl and curcumin were procured from Yucca Enterprises, Mumbai

Instruments and apparatus:

The spectrophotometric measurements were carried out on a Shimadzu-ultraviolet (UV) 1800 spectrophotometer, matched quartz cells 1 cm and UV probe 2.61software was used for all spectral measurements.

Preparation of standard solutions

Accurately weighed 10 mg of quercetin, berberine HCl and curcumin standards were transferred in 10 ml volumetric flask separately and dissolved in 5 ml methanol. The flasks were shaken and volume was made up to mark with Methanol to give solutions containing 1000 µg/ml quercetin,

berberine HCl and curcumin, respectively. From these stock solutions, 2.5 ml aliquots were transferred into 25 ml volumetric flasks and diluted up to mark with phosphate buffer pH 7.4 solutions to get working standard solution containing concentration of quercetin, berberine HCl and curcumin of 100µg/ml respectively.

Selection of analytical wavelength

Solutions of quercetin, berberine HCl and curcumin were prepared in phosphate buffer pH 7.4 by appropriate dilution and spectrums were recorded between 200-800 nm.

The absorption spectra of the solutions prepared at different concentrations of quercetin (2-20 µg/ml), berberine HCl (0.2-10 µg/ml) and curcumin (2-20 µg/ml) were recorded.The double divisor value in various concentrations were calculated to select wavelength range analysis.

Method validation

The proposed analytical method was validated as per the International Conference on Harmonization (ICH) guidelines Q2 (R1).

The developed method was validated for linearity, repeatability, precision, accuracy, limit of detection and limit of quantification. To check the accuracy of the proposed method recovery studies were carried out by spiking 80%, 100% and 120% of standard concentration. The intra-day and inter-day precision studies were ascertained by estimating the responses of three quality control (QC) standards in triplicates under same experimental conditions three times on the same day and on three different days and precision was expressed as %RSD.

Estimation of drugs in the polyherbal formulation

About 0.5gm of gel was dissolved in 10 ml of phosphate buffer pH 7.4 solution and transferred to 50ml volumetric flasks and an appropriate dilution was made with the same buffer solution. The resulting solution was then filtered with 0.45 µm membrane filters before subjecting the solution to spectrophotometric analysis. Drug content was determined from the standard curve of quercetin, berberine HCl and curcumin, respectively.

Results and discussion

Selection of analytical wavelength

Selection of analytical wavelength was carried out by dividing absorption spectra of solutions at different concentrations using the sum of the absorption spectra of solutions of berberine HCl + curcumin (2 µg/ml each in diluents), quercetin+ curcumin (2µg/ml each in diluents), and quercetin+ berberine HCl (2µg/ml each in diluents), respectively, for the determination of quercetin, berberine HCl, and curcumin as double divisor to get the ratio spectra and their first derivatives were plotted with delta lambda 20 nm and scaling factor 5.0. The divided and derivatized spectra's showed maximum and minimum wavelengths are shown in Fig 1-3. The wavelengths 355.11 nm, 264.68 nm, and 399.15 nm were selected for analysis of quercetin, berberine HCl, and curcumin, respectively.

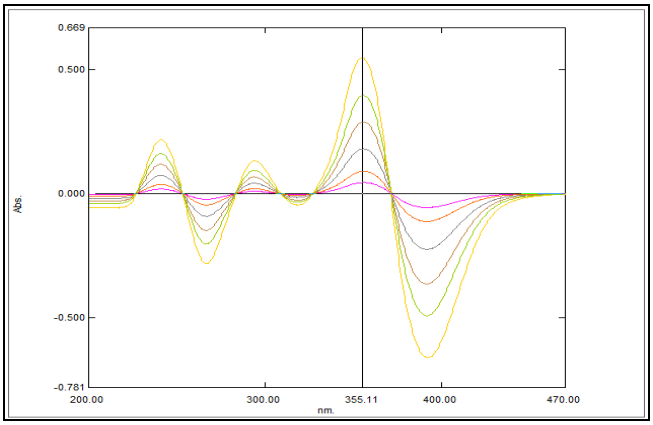

Fig 1: Overlay first derivative ratio spectra of quercetin at 355.11 nm (berberine HCl 2 µg/mL + curcumin 2 µg/mL used as double divisor)

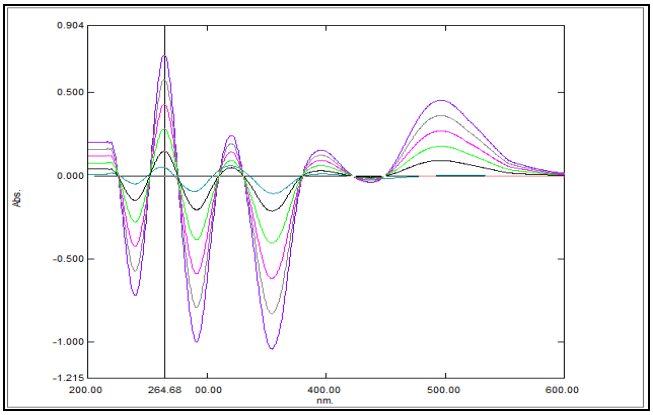

Fig 2: Overlay first derivative ratio spectra of berberine HCl at 264.68 nm (quercetin 2 µg/mL + curcumin 2 µg/mL used as double divisor)

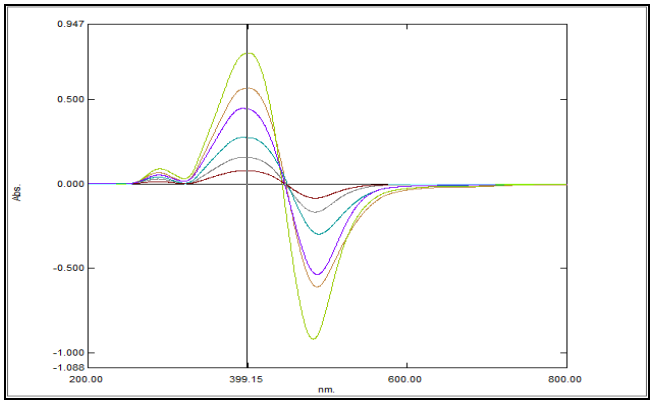

Fig 3: Overlay first derivative ratio spectra of curcumin at 399.15 nm (berberine HCl 2 µg/mL + quercetin 2 µg/mL used as double divisor)

Linearity

The linear regression data for calibration curves showed good linear relationship over the concentration range 2-20 µg/ml for quercetin, 0.2-10 µg/ml for berberine HCl and 2-20 µg/ml for Curcumin. Calibration curve of quercetin, berberine HCl and curcumin are shown in Fig. 4, 5, 6.

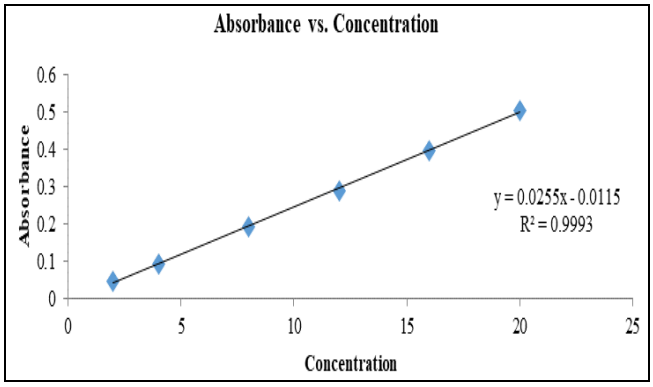

Fig 4: Calibration curve of Quercetin at 355.11nm

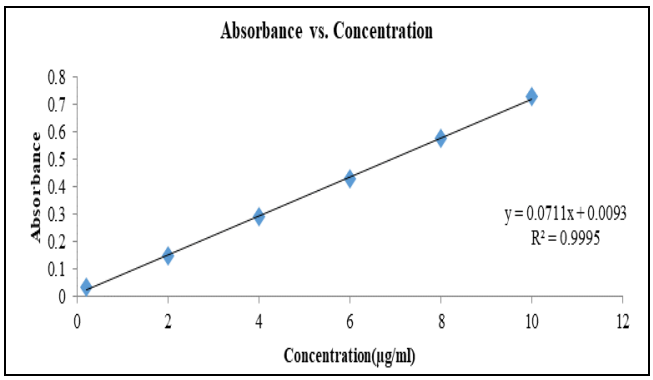

Fig 5: Calibration curve of Berberine HCl at 264.68 nm

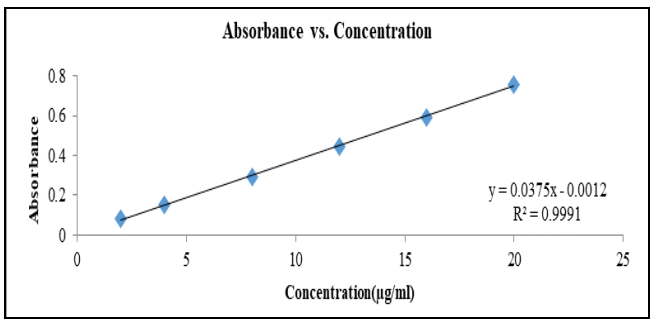

Fig 6: Calibration curve of Curcumin at 399.15 nm

Repeatability

Relative standard deviation (% RSD) for repeatability was found to be 0.83%, 0.40%and 0.61% for quercetin, berberine HCl and curcumin respectively. %RSD value was found to be less than 2. Results of repeatability of method are shown in Table 1.

Table 1: Repeatability studies

Drug	Conc taken (µg/ml) (n=6)	Conc found (µg/ml)	%RSD
Quercetin	4	3.95±0.033	0.83
Berberine HCl	4	3.92±0.015	0.4
Curcumin	4	3.99±0.024	0.61

Precision

The % RSD values were found to be less than 2 that indicate this method is precise for the determination of all 3 drugs in the formulation. The result shows reproducibility of assay. Results of intraday and inter day precision of method are shown in Table 2.

Table 2: Precision studies

Drug	Concentration Taken (µg/ml) (n=3)	Intraday Precision		Interday Precision	
		Conc found (µg/ml)	%RSD	Conc found (µg/ml)	%RSD
Quercetin	4	3.89±0.061	1.57%	3.95±0.061	1.55%
	8	7.87±0.083	1.06%	7.81±0.135	1.73%
	12	11.82±0.140	1.19%	11.77±0.110	0.93%
Berberine HCl	4	3.91±0.021	0.53	3.91±0.020	0.51%
	8	5.91±0.040	0.68%	5.89±0.036	0.61%
	12	7.93±0.051	0.65%	7.95±0.021	0.26%
Curcumin	4	4.04±0.066	1.62%	4.02±0.070	1.75%
	8	7.62±0.081	1.06%	7.70±0.081	1.05%
	12	11.79±0.099	0.84%	11.73±0.131	1.12%

Accuracy

The mean recoveries were found in range of 98.66 – 99.23%, 99.67 – 100.56 % and 98.28 – 99.54 % quercetin, berberine HCl and curcumin, respectively. The results of accuracy studies are shown in Table 3.

Table 3: Accuracy

Drug	Concentration of sample taken(µg/ml) (n=3)	Concentration of standard added (µg/ml) (n=3)	Total Conc. (µg/ml)	Amount Recovered Mean	%Recovery Mean
Quercetin	8	6.4 (80%)	14.4	14.28	99.16
	8	8 (100%)	16	15.88	99.23
	8	9.6 (120%)	17.6	17.36	98.66
Berberine HCl	4	3.2 (80%)	7.2	7.24	100.56
	4	4 (100%)	8	7.97	99.67
	4	4.8(120%)	8.8	8.81	100.11
Curcumin	8	6.4 (80%)	14.4	14.33	99.54
	8	8 (100%)	16	15.84	98.82
	8	9.6 (120%)	17.6	17.3	98.28

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD was found to be 0.1939µg/ml, 0.0325µg/ml and 0.0150µg/ml for quercetin, berberine HCl and curcumin respectively. The LOQ was found to be 0.5875µg/ml, 0.0985µg/ml and 0.0454µg/ml for quercetin, berberine HCl and curcumin respectively.

Table 4: Summary for Validation Parameters

Sr. No.	Validation Parameters	Result obtained		
		Result for Quercetin	Result for Berberine HCl	Result for Curcumin
1.	Wavelength	355.11 nm	264.68 nm	399.15nm

2.	Beer's law range (µg/ml)	2-20	0.2-10	2-20
3.	Slope	0.0255	0.0711	0.0375
4.	Intercept	- 0.0115	0.0093	- 0.0012
5.	Correlation coefficient	0.9993	0.9995	0.9991
6.	Repeatability (n=6) (%RSD)	0.83%	0.40%	0.61%
Precision (% RSD)				
7.	Intraday (n= 3)	1.06%-1.57%	0.53%-0.68%	0.84%-1.62%
	Interday (n= 3)	0.93%-1.73%	0.26%-0.61%	1.05%-1.75%
8.	Accuracy (% Recovery)	98.66-99.23%	99.67%-100.56%	98.28%-99.54%
9.	Limit of detection(µg/ml)	0.1939	0.0325	0.0150
10.	Limit of quantification(µg/ml)	0.5875	0.0985	0.0454

Table 5: Estimation of drugs in the polyherbal formulation

Drugs	% Drug content
Quercetin	99.89±0.38
Berberine HCl	101.03±0.54
Curcumin	100.75±0.25

Conclusion

The developed double divisor ratio derivative spectrophotometry method was found to be accurate and precise for determination of quercetin, berberine HCl and curcuminoids in polyherbal gel formulation without prior separation and can be easily applied for routine analysis.

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