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Qualitative and quantitative evaluation study along with method development and validation for UV spectrophotometric analysis of *Chelidonium majus* L. extract

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Abstract
In this study, a hydroalcoholic solvent system was used to prepare *Chelidonium majus* L extract. Prepared extract was undergone qualitative and quantitative evaluations. Thin layer chromatography was done by applying different solvent systems to acquire knowledge on separation of components. For further analysis related to the plant extract, a suitable UV spectrophotometric method was developed and validated. With due diligence of WHO and ICH guideline Q2(R1) on validation of analytical procedure, cardinal and prime attributes like linearity, range, precision, accuracy, system suitability, robustness, solution stability were analysed. Qualitative evaluations suggested prepared extract contain alkaloid, flavonoid, phenolics, carbohydrates, proteins, saponin, phytosterol etc. Separation process of pure component needed further in depth analysis. Developed UV method was suitable, accurate, precise and robust with the change in laboratory and instrument.

Keywords: Extract, evaluation, thin layer chromatography, UV, validation, stability

Introduction
Chelidonium majus L. is a medicinal plant belonging to family *Papaveraceae* and genus *Chelidonium*. It is also known as greater celandine, swallow wort, swallow herb etc. This plant is herbaceous, perennial and a natural habitat of Europe and Asia. It contains mainly isoquinoline alkaloids like chelidonine, chelerythine, berberine, protopine, coptisine, sanguinarine etc. It also contains chelidonic acid, caffeic acid, p-coumaric acid, ferulic acid etc. This is a plant whose medical pertinence goes back to the time of Roman Empire. During the time of Roman Empire in Europe, Roman scholar Pliny the elder first mentioned about its healing power. As folk medicine in Europe, *Chelidonium majus* L. was used to treat eye diseases, skin ulcers, jaundice, liver disease, gall stones, digestive tract parasites, bronchitis, asthma etc. In recent years many researches were done which showed *Chelidonium majus* L. extract exhibited antimicrobial activity [1, 2], anti fungal activity [3-5], antiviral activity [6-8], anti inflammatory activity [9], antioxidant activity [10-12], antihepatotoxic activity [13-15], choleretic activity [16], anticancer activity [17-19].

Validation is a process, executed to ensure that a process or a specific method will generate results which are within predetermined desired limits and in agreement with the desired quality. Validation should be a documented procedure. In industry, there is always a documented format and protocol to effectively carry out the whole procedure. Analytical method validation is an integral part of the pharmaceutical industry. A method for analysis should always be validated to check that a particular analytical method is affected or remain unaffected by the influence of instruments, environmental conditions, and change in analysts, preparation techniques, and quality of chemicals or reagents. In a whole it can be said that it is a check for stability of a method and preparation. According to ICH Harmonized Tripartite Guideline on validation of analytical procedures Q2(R1) and World Health Organization guidelines on analytical method validation, typical characteristics of an analytical procedure are specificity, linearity, range, accuracy, precision, robustness, limit of detection, limit of quantitation [20, 21]. Both ICH and WHO guidelines followed the same process for each parameter.

In this research work, qualitative and quantitative evaluations of the extract were done. Thin layer chromatographic analysis was carried out to obtain some insight on separation of possible components of the extract. A UV spectrophotometric method was developed for analysis of *Chelidonium majus* L. extract in water. Simultaneously analytical method was validated. Here in this work, we carried out linearity, range, accuracy,

accuracy, precision, robustness studies. In precision analysis, intraday, interday, repeatability were executed. Robustness was analyzed by changing laboratory and equipment. Also, solution stability study and another seven day stability study (absorbance recorded on the first day and on the seventh day) were performed to ensure stability of preparation. These validated analytical procedures will be useful as well as essential for ensuring stability and quality of future drug development stretch.

Materials and methods

Materials

Chelidonium majus L. was purchased as dried whole herb from Associated Traders, Kolkata. Glacial acetic acid (Thermo Fisher Scientific Pvt. Ltd.), diethylamine (E. Merck India Limited), butanol, formic acid, ethyl acetate, toluene (Merck Specialities Private Limited) were solvents used in mobile phase. Silica gel GF254 (Merck Life Sciences Private Limited) was used as stationary phase in thin layer chromatography. Whatman filter (GE Healthcare Life Sciences) was used to remove any particulate matters which could interfere with the analysis. All the other chemicals were of analytical grade and used as received. In this work all the UV analysis was done in Thermo Spectronic UV-1 double beam spectrophotometer and sonication was done using Fast Clean Ultrasonic Cleaner (Enertech Electronics Private Limited). Double distilled water was used all over the whole work.

Extract preparation

Chelidonium majus L. was extracted in 44% v/v ethanol by maceration process. Dried and grinded whole herb was soaked in solvent for seven days with intermediate stirring. Then it was filtered and alcohol was recovered. Water was evaporated at 40°C in a water bath to get a highly concentrated, deep brownish-black liquid which was stored in refrigerator at 4°C until use.

Determination of solid content

5 ml of the concentrated extract was taken in a previously dried and weighed watch glass. It was evaporated to dryness on a water bath. Afterthat it was placed in a hot air oven at 40°C for 6 h and then in desiccator for 30 min. Weight of the watch glass was taken immediately after that. Watch glass was kept in hot air oven again and process was repeated until two consecutive weights were same. Amount of solid present per ml of the solvent was calculated by the following formula –

Concentration (mg/ml) = (Weight of watch glass containing dried solid matter – Weight of empty watch glass) / Volume of extract taken in watch glass

Qualitative and quantitative analysis

Extract was subjected to different qualitative tests for detecting presence of alkaloids (Dragendorff's test, Wagner's test, Hager's test & Mayer's test), glycosides (Borntrager's test & Legal's test), phenolic compounds (Ferric chloride test & Lead acetate test), flavonoids (Lead acetate test, Alkaline reagent test and Shinoda test), phytosterols (Libermann-Buchard test), carbohydrates (Fehling's test, Barfoed test, Benedict test & Molish test), fixed oils (spot test), saponins (foam test), proteins (Ninhydrin test & Biuret test), gums etc [22]. Quantitative tests were done by following specific methods as per WHO quality control methods for herbal materials [23].

Thin layer chromatography

Different solvent systems were used to obtain a little bit of clear idea on separation of alkaloids or other components of extract by thin layer chromatography. List of the solvent systems used were depicted in Table 1. Here butanol, glacial acetic acid and water in the ratio of 4:1:1 was used for thin layer chromatography following the process described in French Pharmacopoeia 2002 for authentication procedure [24]. After development, plates were air dried and observed under ultra violet light at 365 nm. Developed fluorescent spots were observed and R_f value of each spot was calculated.

Table 1: List of TLC Solvent Systems & Ratio

Solvent System	Ratio
Toluene: Ethyl acetate: Diehylamine	7:2:1
Toluene: Ethyl acetate: Formic acid	5:4:1
Butanol: Glacial acetic acid: Water	4:1:1, 8:1:1

Method development and validation

Here in this method development process water was selected as solvent system for analysis. The rationale for selecting this solvent system was endowing systematization in case of further future works. All stock and test solutions were degassed in a bath sonicator to remove bubbles formed during preparation and filtration. All of the test solutions were prepared in triplicates to maintain the uniformity and accuracy throughout the entire process. Above mentioned processes were essential to obtain better results.

Preparation of standard stock and test solutions

A stock solution of 1 mg/ml was prepared by dissolving the extract in double distilled water. From the above mentioned stock solution, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml of solution were withdrawn to make test solutions of concentration 10, 20, 40, 60, 80 and 100 µg per ml by the addition of double distilled water to make up the volume.

Determination of UV absorption

All the zones in each TLC were scrapped out, dispersed in water and filtered through whatman filter paper. Each of the filtrate collected were scanned in UV-visible spectrophotometer (200-400 nm) to observe if any absorption maxima could be obtained for any of the zone.

Linearity and range

In case of determining linearity, stock and test solutions were prepared following the same process as calibration curve plotting technique. All of the test solutions were prepared in triplicates. Calibration curve was plotted using six concentrations in the range of 10-100 µg/ml. Also correlation coefficient along with equation for regression line was obtained.

System suitability

System suitability is a parameter, determined to evaluate the system which is being used to carry out the analysis. System suitability was measured before each operation. Six replicate reading of test solution of 100 µg/ml concentration was documented. From mean and standard deviation, %RSD value was calculated.

Precision

Repeatability was carried out by determining absorbance of three concentrations. Each concentration was prepared in triplicates. Intermediate precision study was executed by measuring absorbance of all of test solutions in same day and

different day. All of the test solutions were prepared in triplicates.

Accuracy
Accuracy study was done at 50%, 100% and 150% level. To 1 ml solution of 10 µg/ml, 1 ml solution of 10, 20, 30 µg/ml was added consecutively. Study at each level was carried out in triplicates. Mean percentage recovery was calculated for each level.

Robustness
Robustness was carried out by measuring absorbance of all of test solutions by changing laboratory and equipment. All of the test solutions were prepared in triplicates.

Solution stability
This analysis was carried out by measuring absorbance of the test solution with known concentration at two hour interval up to 8 hour. All of the test solutions were prepared in triplicates. Limit was calculated by following formula-

Limit = [(Absorbance of initial – Absorbance at different times)/Absorbance of initial] × 100

Extract stability over seven day period
It was carried out by measuring absorbance of all of test solutions at first day and same procedure was repeated at seventh day. All of the test solutions were prepared in triplicates. From mean and standard deviation values, %RSD values were obtained.

Results and discussion

Qualitative phytochemical analysis
Solid content of the extract was found to be 134 mg/ml. Qualitative phytochemical analysis showed presence of alkaloid, glycoside, carbohydrate, protein, phenolic compound, flavonoid, phytosterol, saponin, fixed oil, gum. Detailed results were shown in Table 2. Mayer's test resulted in formation of precipitate without the cream or white colour. During testing for presence of carbohydrates, only Molish test gave positive result. Fehling's test also yielded precipitate but not in test specific red colour. Barfoed and Benedict tests produced negative results. Though tests were repeated, but results were same. Since Molish test generally gives positive result for mono, di and polysaccharides, analyzing the results it was concluded that extract contained carbohydrates but may be devoid of reducing sugars or monosaccharides. For determining presence of flavonoid, among three of the tests, alkaline reagent test and shinoda test both gave negative results although lead acetate gave positive result. It can be concluded from the results that extract was either devoid of flavonoids or presented in such small quantity which can't be detected by qualitative tests.

Table 2: Qualitative phytochemical analysis

Type of Component	Process/Name of the test	Result (Positive/Negative/Inconclusive)
Alkaloids	Dragendorff's test	Positive
	Mayer's test	Inconclusive (Precipitate appears but not white or creamy in colour)
	Wagner's test	Positive
	Hager's test	Positive
Carbohydrates	Molish test	Positive
	Fehling's test	Inconclusive (Precipitate appears but not red in colour)
	Barfoed test	Negative
	Benedict test	Negative
Glycosides	Borntrager's test	Negative
	Legal's test	Negative
Saponins	Foam test	Positive
Proteins and Amino acids	Ninhydrin test	Positive
	Biuret test	Positive
Phytosterols	Liberman – Burchard test	Positive
Fixed Oils and Fats	Spot test	Negative
Phenolic Compounds	Ferric chloride test	Positive
	Lead acetate test	Positive
Flavonoids	Lead acetate test	Positive
	Alkaline reagent test	Negative
	Shinoda test	Negative
Gums and Mucilage	Addition of absolute alcohol to the extract	Positive

Quantitative phytochemical analysis
Results of quantitative analysis were shown in Table 3. According to European Pharmacopoeia 5.0, results of ash value and moisture content of *Chelidonium majus* L. are within the limits [25]. It was seen that water soluble extractive value was more when dried matter was boiled with water than keeping it immersed in room temperature. Foaming index was less than 100 indicating presence of saponins which was also confirmed in qualitative test.

Table 3: Quantitative determinations

Name		Result
Ash value	Total ash	12.75%
	Water soluble ash	3.5%
	Acid insoluble ash	1%
Moisture Content		10%
Water soluble extractive value	Cold	13%
	Hot	19%
Foaming index		<100

Thin layer chromatography

Solvent system containing toluene, ethyl acetate, diethylamine in the ratio of 7:2:1, resulted in appearance of two spots (Fig 1. A) a large bright yellow spot (LY) and a blue spot (B). Solvent system toluene, ethyl acetate, formic acid in the ratio of 5:4:1 resulted in appearance of six spots (Fig 1. B). From top to bottom spots were slightly intense large pink spot (LP), small pink spot (SP-1), blue spot (B), and second small pink spot (SP-2), white spot (W). Separation of yellow zone was not occurred using this solvent system. A slightly separated bright yellow zone was retained in the origin point.

TLC solvent system comprised of butanol, glacial acetic acid and water in the ratio of 4:1:1 (Fig 1. C) showed appearance of five spots - a lower blue spot (LB), a lower greenish yellow spot (LGY), a intense and bright yellow spot/zone (IY), a upper greenish yellow spot (UGY), an upper blue spot (UB) and a light red spot (LR). Lower greenish yellow (LGY) and upper greenish yellow (UGY) spots were not very much prominent in the picture. Appearance of zones was in order as described in French Pharmacopoeia 2002 [24] with presence of an upper greenish yellow zone which is characteristic of berberine above the bright yellow zone. A red brown zone was absent in this TLC analysis. This may be due to absence of the component. In this work, whole plant which was used for extraction contained largely aerial parts comparing to the proportion of root part. Also there were reports of seasonal variability in quantity of components of *Chelidonium majus* L. [12, 26]. So, in this case may be the aerial parts were devoid of the component led to absence of that zone. This solvent system led to separation of the intense bright yellow part prominently comparing other solvent systems. But there was occurrence of tailing which may be due to high concentration of aqueous part of extract (used for TLC) or presence of glacial acetic acid and water which were polar components. So, TLC was again carried out with the same solvent system only ratio was changed to 8:1:1 (butanol: glacial acetic acid: water). It was observed that tailing was decreased satisfactorily (Fig1. D) and it caused separation of yellow zone (IY) and lower greenish yellow zone (LGY) more prominently than previous ratio (4:1:1). New white zone (W) was visible on top of intense yellow zone (IY). But UB, P, LB zones were absent. It was seen that most of the solvent system showed separation of pink and blue spot with higher R_f values. Toluene, ethyl acetate and formic acid led to separation of blue and pink parts more distinctly. Retention factor for each corresponding spots were given in Table 4. Observing the area of each zone in each of the TLC procedure, it can be easily observed that the intense yellow zone (IY) was appeared in a larger extent with deep intensity in both butanol, glacial acetic acid and water solvent system and also with little bit lighter intensity in toluene, ethyl acetate, diethylamine solvent system.

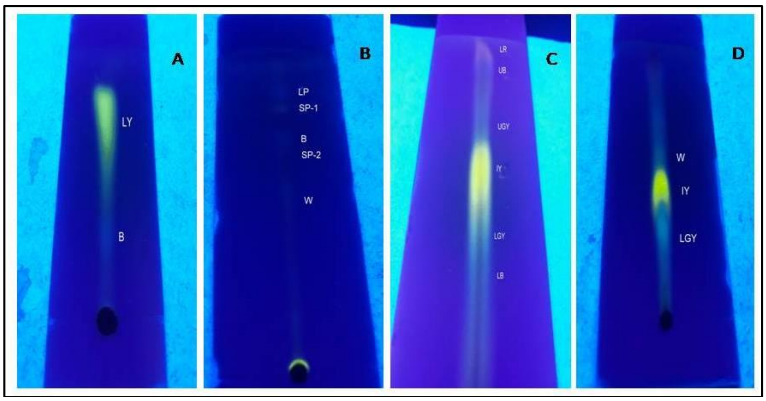

Fig 1. TLC plates under UV 365 nm, A: toluene: ethyl acetate: diethylamine (7:2:1), B: tolune: ethyl acetate: formic acid (5:4:1), C: butanol: glacial acetic acid: water (4:1:1), D: butanol: glacial acetic acid: water (8:1:1)

Table 4: Spots with R_f values

Solvent System & Ratio	Spots & Corresponding Retention Factor
Toluene: Ethyl acetate: Diehylamine (7:2:1)	LY (0.73), B (0.22)
Toluene: Ethyl acetate: Formic acid (5:4:1)	LP (0.93), SP-1 (0.85), B(0.71), SP-2 (0.68), W (0.52)
Butanol: Glacial acetic acid: Water (4:1:1)	LB (0.18), LGY (0.23), IY (0.44), IGY (0.53), UB (0.84), LR (0.94)
Butanol: Glacial acetic acid: Water (4:0.5:0.5)	LGY (0.21), IY (0.41), W(0.50)

UV absorption maxima

Scanning of the scrapped intense yellow (IY) zone collected from TLC plate using solvent system butanol, glacial acetic acid and water in the ratio of 8:1:1, gave lambda max of 272 nm in water (Fig 2.). Appearance of a single peak in UV scanning procedure was also indicating to the presence of a pure unknown compound. Due to the reason, this wavelength was selected for further analytical determinations. Though other TLC zones were unable to show any absorption maxima in UV range of 200-400 nm.

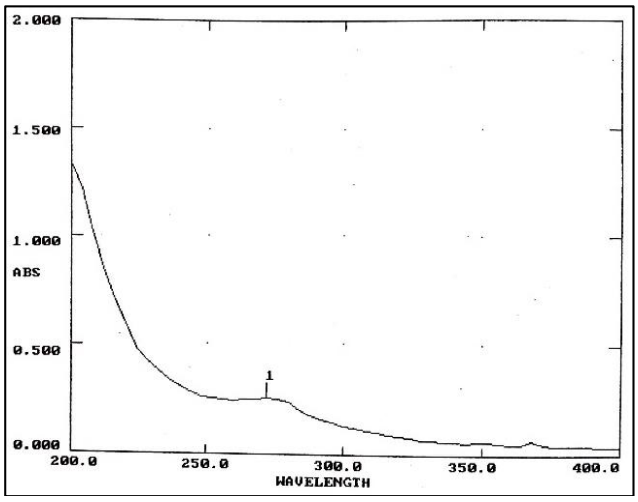

Fig 2: UV spectrum for determination of lambda max

Calibration curve

Absorbance versus concentration plot yielded the calibration curve (Fig 3.) Data was shown in Table 5. In water, correlation coefficient was 0.999, regression equation was y = 0.006x (Fig 3.).

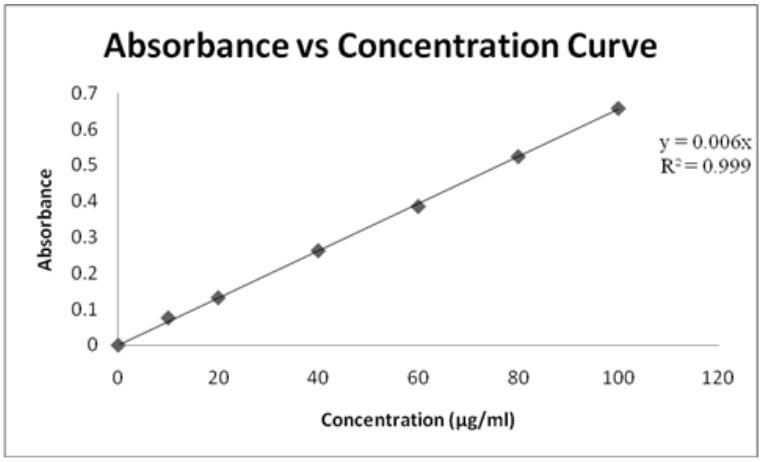

Fig 3: Calibration curve

Table 5: Table for plotting calibration curve

Sl. No.	Conc. (µg/ml)	Absorbance			Average Absorbance ± SD
1	10	0.076	0.078	0.075	0.07633 ± 0.00153
2	20	0.132	0.133	0.133	0.13267 ± 0.00058
3	40	0.261	0.264	0.265	0.26333 ± 0.00208
4	60	0.389	0.385	0.386	0.38667 ± 0.00208
5	80	0.527	0.524	0.524	0.52500 ± 0.00173
6	100	0.66	0.659	0.659	0.65933 ± 0.00058

Linearity

Linearity study data was shown in Table 6. Six concentrations from 10 – 100 µg/ml were used to plot the calibration curve (Fig 4.). Correlation coefficient was 0.999 and regression equation was y = 0.006x. It was observed that absorbance versus concentration curve was linear in the range of 10 – 100 µg/ml of test concentration. This specific UV method represented excellent linearity in the suitable range.

Table 6: Table for Linearity Study

Conc.(µg/ml)	Absorbance			Average	SD	% RSD
10	0.073	0.074	0.071	0.07267	0.00153	2.1021
20	0.135	0.134	0.138	0.13567	0.00208	1.5344
40	0.268	0.263	0.27	0.26700	0.00361	1.35039
60	0.385	0.386	0.382	0.38433	0.00208	0.54163
80	0.526	0.523	0.529	0.52600	0.00300	0.57034

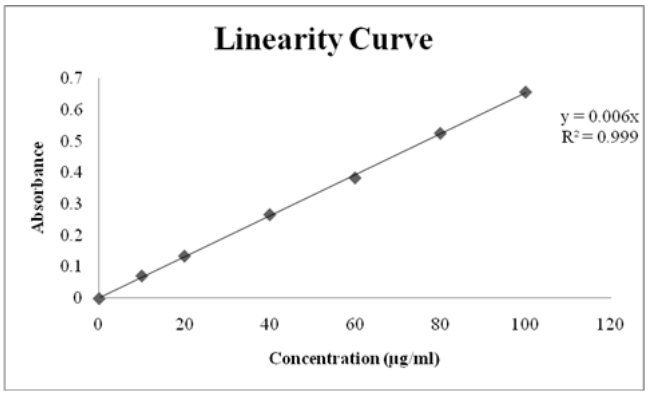

Fig 4: Linearity curve

System suitability

From six replicate readings of test solution with concentration of 100µg/ml, %RSD was calculated and it was 0.07864 (Table 7). Results illustrated suitability of the UV spectrophotometric system.

Table 7: Table for system suitability study

Sl. No.	Absorbance
1	0.657
2	0.657
3	0.656
4	0.657
5	0.657
6	0.656
Average	0.65667
SD	0.00052
%RSD	0.07864

Accuracy

Results of accuracy study were depicted in Table 8. Percentage recovery was 99.40-104.10%. Results of percentage recovery indicated that the method was accurate and producing results with good accuracy.

Table 8: Table for Accuracy

Sl. No.	Initial amount (µg/ml)	Amount added (µg/ml)	Final Conc. (µg/ml)	Obtained avg. conc. (µg/ml)	Mean % Recovery
1	10	10 (50%)	10	10.41	104.10
2	10	20 (100%)	15	14.91	99.40
3	10	30 (150 %)	20	20.07	100.35

Precision

Repeatability study was carried out by recording absorbance of 20, 40, 60 µg/ml in triplicates and %RSD value was calculated (Table 9). %RSD was less than 2 and all the results indicated repeatability of the method. Interday precision was carried out for three days. Each day absorbance of test

solutions (10 – 100 µg/ml) was recorded. For each day, %RSD values were calculated (Table 10). In case of intraday precision, %RSD values at 9 am and 5 pm were calculated. Results were shown in Table 11. It was observed that both interday and intraday studies produced precise results with %RSD values less than 4 in each case. So it can be justified that the method yielded repeatable and precise results.

Table 9: Table for repeatability study

SL. No.	Conc. (µg/ml)	Average absorbance	SD	% RSD
1	20	0.135	0.001	0.74074
2	40	0.26633	0.00058	0.21678
3	60	0.38467	0.00289	0.75046

Table 10: Table for interday precision study

SL. No.	Conc. (µg/ml)	1st Day			2nd Day			3rd Day		
		Avg.	SD	%RSD	Avg.	SD	%RSD	Avg.	SD	%RSD
1	10	0.07067	0.00252	3.56124	0.072	0.00200	2.77778	0.07533	0.00153	2.02769
2	20	0.13467	0.00115	0.85745	0.13467	0.00208	1.54579	0.13467	0.00115	0.85745
3	40	0.26700	0.00100	0.37453	0.26767	0.00289	1.07849	0.26433	0.00153	0.57788
4	60	0.38500	0.00200	0.51948	0.38467	0.00208	0.54116	0.38367	0.00208	0.54257
5	80	0.52367	0.00289	0.55126	0.52533	0.00306	0.58155	0.52567	0.00252	0.47875
6	100	0.65500	0.00100	0.15267	0.65800	0.00200	0.30395	0.65800	0.00173	0.26323

Table 11: Table for intraday precision study

SL. No.	Conc. (µg/ml)	9 AM			5 PM		
		Avg.	SD	%RSD	Avg.	SD	%RSD
1	10	0.07267	0.00115	1.58904	0.07167	0.00252	3.51155
2	20	0.13533	0.00252	1.85957	0.13433	0.00153	1.13712
3	40	0.27	0.001	0.37037	0.267	0.00265	0.99092
4	60	0.38133	0.00404	1.05982	0.38233	0.00153	0.39953
5	80	0.52433	0.00306	0.58265	0.52433	0.00115	0.22022
6	100	0.65767	0.00416	0.63305	0.65633	0.00289	0.43983

Robustness

Absorbance of same test solutions (10 – 100 µg/ml) was recorded in different instrument. Test solutions (10 – 100 µg/ml) were prepared in two different laboratories and absorbance of those was recorded. %RSD was calculated in each process and results were interpreted in Table 12, 13. %RSD was less than 4 in each case and results represented robustness of the method which was remained unaffected by the change of equipment and laboratory.

Table 12: Table for robustness data with change in equipment

SL. No.	Conc. (µg/ml)	Equipment-1			Equipment-2		
		Avg.	SD	%RSD	Avg.	SD	%RSD
1	10	0.07400	0.00173	2.34061	0.07633	0.00231	3.02542
2	20	0.13600	0.00265	1.94541	0.13833	0.00208	1.50482
3	40	0.26200	0.00100	0.38168	0.26300	0.00100	0.38023
4	60	0.38267	0.00231	0.60350	0.38733	0.00208	0.53744
5	80	0.52400	0.00200	0.38168	0.52233	0.00321	0.61542
6	100	0.65233	0.00231	0.35402	0.65333	0.00231	0.35348

Table 13: Table for robustness data with change in laboratory

SL. No.	Conc. (µg/ml)	Laboratory-1			Laboratory-2		
		Avg.	SD	%RSD	Avg.	SD	%RSD
1	10	0.07300	0.00000	0.00000	0.07467	0.00115	1.54647
2	20	0.13367	0.00208	1.55736	0.13200	0.00173	1.31216
3	40	0.26567	0.00058	0.21732	0.26500	0.00361	1.36059
4	60	0.38333	0.00321	0.83858	0.38500	0.00173	0.44988
5	80	0.52133	0.00115	0.22149	0.52467	0.00058	0.11004
6	100	0.65633	0.00252	0.38343	0.65900	0.00200	0.30349

Solution stability

Absorbance of test solution (100µg/ml) was recorded in every two hours up to eighth hour. Results were interpreted in Table 14. It was seen from the absorbance data that solution was very much stable over a period of 8 hour.

Table 14: Table for solution stability study

SL. No.	Time (h)	Absorbance	Limit
1	0	0.660	0
2	2	0.660	0
3	4	0.659	0.151
4	6	0.660	0
5	8	0.659	0.151

Extract stability over seven day period

Absorbance of test solutions (10 – 100 µg/ml) was recorded at first day and at seventh day. %RSD was calculated and results were interpreted in Table 15. Absorbance results of first day and seventh day depicted stability of the stored extract over seven day period. Also %RSD values were less than 4 for each concentration.

Table 15: Table for stability study over seven day period

SL. No.	Conc. (µg/ml)	Day-1			Day-7		
		Avg.	SD	%RSD	Avg.	SD	%RSD
1	10	0.07233	0.00115	1.59636	0.07500	0.00265	3.52767
2	20	0.13133	0.00289	2.19803	0.13467	0.00058	0.42873
3	40	0.26333	0.00208	0.79051	0.26433	0.00289	1.09209
4	60	0.38367	0.00153	0.39814	0.38633	0.00252	0.65141
5	80	0.52700	0.00000	0.00000	0.52500	0.00361	0.68677
6	100	0.65333	0.00153	0.2338	0.65267	0.00208	0.31895

Conclusion

From TLC analysis it can be acquainted that time of collection and part of plant material collected are the two very influencial aspects for identification and isolation of specific components. Though thin layer chromatographic study done in this research work needs further thorough analysis to optimize the separation process of more pure components. This may need further confirmation on the structural elucidation par with the help of advanced analytical instruments but it can be told that a very crucial finding was obtained with TLC procedure and furher scanning of the zones in UV spectrophotometry. Here in this proposed study, a suitable UV method was developed for analysis of *Chelidonium majus* L. extract followed by validation as per ICH Q2 (R1) guideline and World Health Organization guideline on analytical method validation. This method will be suitable for use for future formulation development work using the plant extract. This developed method was proved to be simple to use, accurate, precise, linear, robust alongwith good stability of the analysing solution and good repeatability of the obtained results. It can be said that the developed method can be used in future for further research works and development projects.

Conflict of interest

The authors report no declarations of interest.

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