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Original Article

SIMULTANEOUS METHOD DEVELOPMENT AND VALIDATION OF CHOLINE SALICYLATE AND TANNIC ACID USING RP-HPLC IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: The current investigation was pointed at a completely unique and reliable high-performance liquid chromatographic method for simultaneous quantification of Choline salicylate and Tannic acid.

Methods: Chromatographic separation was achieved on a symmetry C_{18} column (150x4.6 mm, 3.5 µ) using isocratic elution with a buffer containing 1 ml of OPA in l lt of HPLC marked water and acetonitrile within the percentage of 60:40 as a movable phase with a flow rate of 1.0 ml/min at ambient temperature. Analysis was achieved within 15 min over honest linearity within the concentration range from 4-60 µg/ml of choline salicylate and 2.5-37.5 µg/ml of tannic acid. Stress conditions of degradation in acidic, alkaline, peroxide and thermal were studied.

Results: LOD and LOQ were observed as 1.21 µg/ml, 0.758 µg/ml and 4 µg/ml, 2.5 µg/ml of choline salicylate and tannic acid, respectively. Precision and recovery study results were found to be within the suitable limit.

Conclusion: This developed method showed reliable, precise, accurate results under optimized conditions. The method was validated as reported by ICH guidelines. Hence it was evident that the proposed method was suitable for regular analysis and quality control of pharmaceutical preparations.

Keywords: HPLC, Choline salicylate, Tannic acid, Development, Validation

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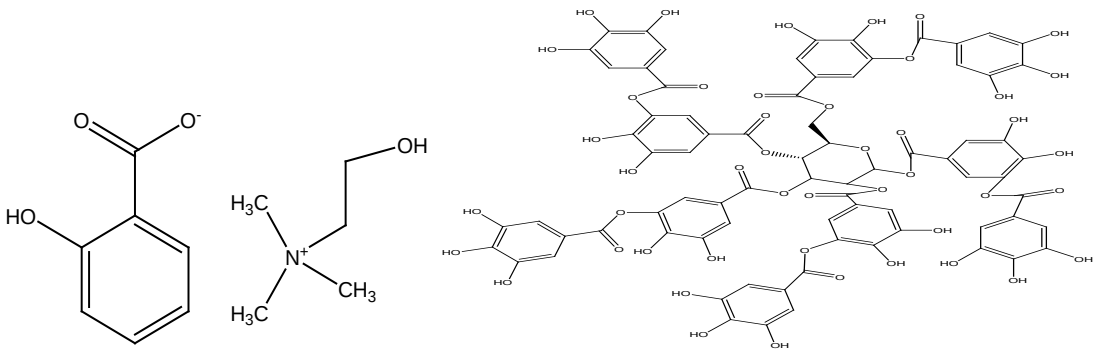
INTRODUCTION

Choline salicylate is used as a pain reliever. It is an anti-inflammatory [1] drug-related to aspirin. In children and adults, choline salicylate is used to treat arthritis and used to reduce swelling and mild-moderate pain. This drug is also used to treat fever [2, 3]. Choline salt of salicylic acid (generally called as choline salicylate), used as an analgesic, anti pyretic [4] and anti rheumatic [5, 6]. It reduces mild to moderate pain, fever and inflammation or swelling. Choline salicylate is powerful in the treatment of gout [7, 8], rheumatic fever [9, 10], rheumatoid arthritis [11, 12] and muscle injuries. To relieve pains associated with tooth growth in infant population, choline salicylate is used as main ingredient in teething gels. Orthodontic [13, 14] devices, cold sores or mouth ulcers.

Tannic acid is a fixed form of tannin, and it is a type of polyphenol. It is a weak acid (pKa around 6) because of the more number of phenol groups present in the structure. Its chemical formula is $C_{76}H_{52}O_{46}$ which corresponds with decagalloyl glucose. In fact it is a composition of polygalloyl glucoses or polyagalloyl glucoses or polygalloyl quinic acid esters with the number of galloyl moieties per molecule ranging from 2 to 12 depending on the plant source used to extract the tannic acid. Commercial tannic acid is usually extracted from plant parts of Tara pods (Caesalpinia spinosa), gall nuts from Rhus semialata [15] or Quercus infectoria or Sicilian sumac leaves (Rhus coriaria). The aim of the study is to separate the pharma ingredients Choline salicylate and Tannic acid by using RP-HPLC.



Choline salicylate **Tannic acid**

Fig. 1: Chemical structures of choline salicylate and tannic acid

Till today there are no HPLC methods were reported in the literature. Hence we developed a method for the simultaneous quantification of Choline salicylate and Tannic acid. The developed HPLC method was utilized for the estimation of the combined drugs by *in vitro* method.

MATERIALS AND METHODS

Chemicals and reagents

Acetonitrile and OPA, water (HPLC grade) were taken from Merck (India) Ltd, Worli, Mumbai, India. All APIs of choline salicyalte and

tannic acid as reference standards were taken from Spectrum Pharma Research Solutions Pvt. Ltd., Hyderabad.

Equipment

An HPLC system (Waters alliance e2695 model) consisting of a quaternary pump, PDA detector-2998, was used. Data processing was performed with Empower software of version 2 [16-20].

Chromatographic conditions

Chromatographic separation was carried out in isocratic mode at room temperature using symmetry C_{18} column (150x4.6 mm, 3.5 µ). A mixture of acetonitrile and 0.1% OPA in 40:60 v/v at a flow of 1 ml/min was used as movable phase. The injection volume was 10 µl and the run time was 10.0 min.

Preparation of buffer

1 ml of OPA was dissolved in 1 l of HPLC grade water and filtered through 0.45 µ filter paper.

Preparation of mobile phase

Acetonitrile and 0.1% orthophosphoric acid were taken in 40:60 ratio and filtered with 0.45 µ filter paper.

Diluent

The above prepared mobile phase solution was used as diluent.

Preparation of standard and quality control samples

Accurately weighed and moved 40 mg of choline salicylate and 25 mg of tannic acid in 100 ml flask and added approximate 70 ml of diluent, sonicated to dissolve it for 30 min. and made with diluent. Further diluted 5 ml of the above-prepared solution to 50 ml with diluent.

Preparation of sample solution

Accurately weigh and moved weight equivalent to 40 mg of choline salicylate and 25 mg of tannic acid sample in 100 ml of flask and added 70 ml of diluent. It was sonicated to dissolve and diluted with diluent. 5 ml of the above stock solution was taken and diluted to 50 ml and filtered through 0.45 µ nylon syringe filter.

RESULTS AND DISCUSSION

The current study was designed to develop a simple, precise and rapid analytical RP-HPLC method, which can be used for the analysis of assay method for simultaneous estimation of choline salicylate and tannic acid in bulk and pharmaceutical dosage form. To optimize mobile phase, various combinations were tried for the separation of choline salicylate and tannic acid. The optimized mobile phase is 0.1% OPA and acetonitrile in the percentage of 60:40 v/v. Detection was carried out in several wavelengths in order to obtain enough sensitivity for the two APIs in smaller proportion (choline salicylate and tannic acid). At last the wavelength 224 nm was selected at which the two drugs showed good absorbance. The flow rate was 1.0 ml/min. The retention time for choline salicylate and tannic acid were 2.050 min, 3.942 min, respectively. The proposed method is validated [21, 22] in accordance with the ICH guidelines with all of the results within the limits. The observation was performed with a total runtime of 5.0 min.

Table 1: Optimized chromatographic conditions

Parameter	Optimized condition
Stationary phase	Symmetry C_{18} (150x4.6 mm, 3.5 µ)
Mobile phase	0.1% OPA: Acetonitrile (60:40)
Injection volume	10 µl
Flow rate	1.0 ml/min
Column temperature	25 °C
Wavelength	224 nm
Run time	5.0 min
Retention time of choline salicylate	2.050 min
Retention time of tannic acid	3.942 min

System suitability

The system suitability was performed by injecting a standard solution containing 40 µg/ml of choline salicylate and 25 µg/ml of tannic acid in six replicates. The results indicate that the system suitability parameter is within the limit [23].

Specificity

There was no interference from blank at the retention time of choline salitylate, tannic acid [24].

Linearity

Linearity was determined by drawing a calibration curve of peak response against their respective concentration. From this calibration curve it was found that the curve was straight line in the range of 4-60 µg/ml of choline salicylate and 2.5-37.5 µg/ml of tannic acid. The regression equations for choline salicylate was Y= 63382.68x+16579.44 (R^2-0.99989) and Y= 53507.68x+10790.28 (R^2-0.99963) for tannic acid respectively [25].

Table 2: Results of system suitability

Parameter	Choline salicylate		Tannic acid	
	Mean	Std dev	Mean	Std dev
Theoretical plate count	4275	39.484	8651	49.918
Tailing factor	1.05	0.005	1.02	0.033
Resolution	-	-	7.72	0.054
Retention time	2.056	0.010	3.948	0.007

mean±SD (n=6)

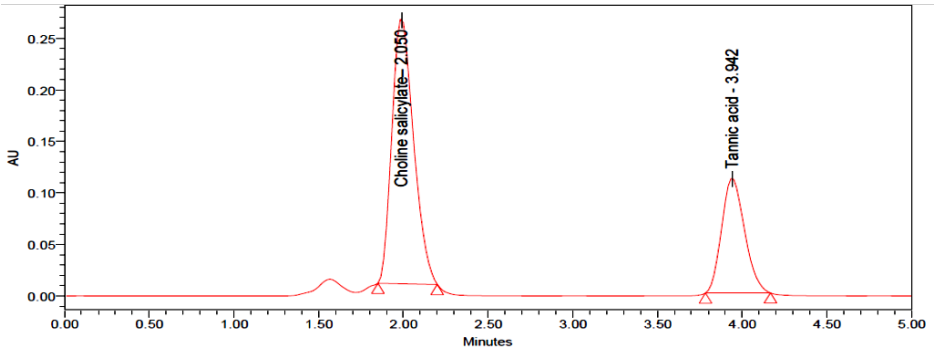


Fig. 2: Chromatogram of system suitability

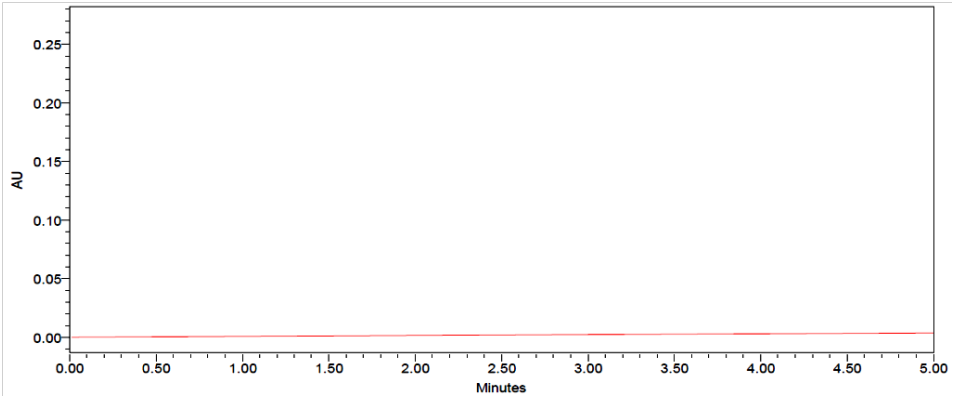
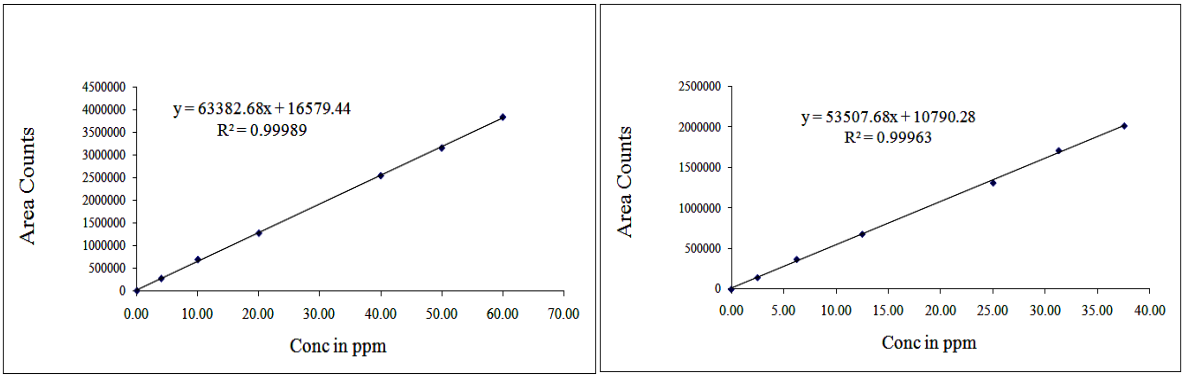


Fig. 3: Chromatogram of blank

Table 3: Results of linearity

S. No.	Choline salicylate		Tannic acid	
	Concentration (µg/ml)	Area	Concentration (ng/ml)	Area
1	4.00	270078	2.50	143686
2	10.00	687392	6.25	368414
3	20.00	1273025	12.50	679383
4	40.00	2544502	25.00	1311917
5	50.00	3160044	31.25	1710677
6	60.00	3843429	37.50	2014838



A **B**

Fig. 4: Calibration plots of (A) Choline salicylate and (B) Tannic acid

Precision

Precision of this method was assessed in terms of intraday (repeatability) and (intermediate precision) variations [26]. The intraday studies were determined by performing six repeated analysis of the sample solution of choline salicylate and tannic acid on the same day under the same experimental conditions. The intermediate precision of the method was carried out in the same laboratory by studying the analysis with different analyst and different instrument. The method is highly precise as %RSD values were found to be<2%. Good recoveries of the drug were obtained at each added concentration, indicating that the method was accurate.

Table 4: Results of method precision

S. No.	Area of choline salicylate	Area of tannic acid
1	2542689	1344264
2	2553130	1351927
3	2505117	1323328
4	2577854	1324891
5	2591109	1355421
6	2514689	1341485
Mean	2547431	1340219
Std. dev	33914.023	13463.999
% RSD	1.33	1.01

n=6

Intermediate precision (Ruggedness)

Table 5: Results of intermediate precision

S. No.	Area of choline salicylate	Area of tannic acid
1	2533420	1316548
2	2574510	1338200
3	2541458	1348243
4	2585241	1326984
5	2519951	1319124
6	2565069	1307808
Mean	2553274	1326151
Std. dev	25519.406	14913.019
% RSD	1.01	1.12

n=6

Accuracy

The accuracy of the method was performed by calculating the recovery experiments at three levels (50%, 100% and 150%). APIs with concentrations 20, 40, 60 µg/ml of choline salicylate and 12.5, 25, 37.5 µg/ml of tannic acid were prepared. The sample solution was introduced three times for each spike level and the assay was carry out as per the test method. The recovery results were close to 100% and also the RSD values were less than±2%. The percentage recovery, mean and relative standard deviation were calculated. Recovery values demonstrated that the method was accurate within desired range.

LOD and LOQ

The LOD concentration for choline salicylate was 1.21 µg/ml and s/n value was 6 and tannic acid was 0.758 µg/ml and s/n value 3. The LOQ concentration for choline salicylate 4.0 µg/ml and its s/n value was 27 and tannic acid 2.5 µg/ml and s/n value was 24.

Robustness

Robustness of the chromatographic method was determined by varying flow rate and mobile phase composition [27]. % RSD was found to be within the acceptable limit.

Table 6: Results of accuracy

Accuracy level	Choline salicylate % recovery		Tannic acid % recovery	
	Mean	Std dev	Mean	Std dev
50	100.6	1.342	100.1	0.806
100	100.1	1.071	100.2	1.534
150	100.5	0.668	100.4	1.455

n=3

Forced degradation

The proposed technique can be used for release and stability studies for effective evaluations and can be considered as the stability-indicating method. The forced degradation [28] study was carried out according to the ICH requirements [29, 30] include acid, base, oxidation, reduction, thermal, photolytic degradation [31, 32]. From the chromatograms it is evident that the selected drugs were stable under the applied stress conditions though the degraded peaks were observed.

Table 6: LOD and LOQ for choline salicylate and tannic acid

Choline salicylate				Tannic acid			
LOD		LOQ		LOD		LOQ	
Concentration	s/n	Concentration	s/n	concentration	s/n	Concentration	s/n
1.21 µg/ml	6	4.0 µg/ml	27	0.758 µg/ml	3	2.5 µg/ml	24

Table 7: Results of robustness

Parameter	% RSD of choline salicylate		% RSD of tannic acid	
	Mean	Std dev	Mean	Std dev
Flow (0.8 ml/min)	100.3	1.015	99.6	1.537
Flow (1.2 ml/min)	99.7	0.971	100.4	1.405
Organic phase (36:64)	100.4	0.794	100.1	1.557
Organic phase (44:56)	99.9	1.45	100.6	1.429

RSD-Relative standard deviation, n=3

Table 8: Results of forced degradation

Stress parameter	% of degradation	
	Choline salicylate	Tannic acid
Acid degradation (1N HCl)	13.9	13.5
Alkali degradation (1N NaOH)	14.5	12.6
Peroxide degradation (30% Peroxide)	15.7	14.3
Reduction degradation (30% sodium bi sulphate)	11.1	10.4
Thermal (sample, 70 °C, 6 H)	2.4	1.1

CONCLUSION

In this study, a novel, rapid, economical, sensitive and easily available HPLC method was developed for the simultaneous estimation of choline salicylate and tannic acid bulk and marketed formulation. The main advantage of this method is no HPLC methods were reported. In this method, shorter run time, low price, accessibility, sensitivity, reliability and reproducibility are used in this process. These properties are significant when it is essential to evaluate a large number of samples. The validation of all the parameters like linearity, accuracy, specificity, robustness, method precision was done and found to be within the appropriate limit. For all the parameters, the RSD values were observed to be less than 2%, which means the method's validity and the outcomes derived by this methodology are in fair agreement. So the prefered method could be easily applied for the normal investigation and the pharmaceutical formulations of choline salicylate and tannic acid in quality control laboratories without any preliminary separation.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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