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Research Article

Validated HPLC-UV method for the determination of Cichorin in *Cichorium intybus* Linn.

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Abstract
A new, simple, sensitive, selective and precise high-performance liquid chromatography (HPLC) method for analysis of Cichorin in *Chicoria intybus* was developed and validated. Separation was achieved using a C_{18} reverse phase column with a mobile phase comprising of water and methanol (HPLC grade) using isocratic flow and flow rate of 1.0 mL min−1 and elution was monitored at 346 nm. The linear regression analysis data for the calibration plot showed good linear relationship with r^2 = 0.991 in the concentration range of 5.3–10.5 ppm with respect to peak area. According to the International Conference on Harmonization (ICH) guidelines the method was validated for specificity, precision, recovery and linearity. Statistical analysis of the data showed that the method is reproducible and selective for the estimation of Cichorin. This is the first such method developed for the quantification of cichorin in *Cichorium intybus*.

Key Words: *Cichorium intybus*, Cichorin, Reverse phase chromatography, HPLC, Cichorin

Introduction
Cichorium intybus L. is a medicinally important plant that belongs to the family *Asteraceae*. The tuberous root of this plant contains number of medicinally important compounds such as inulin, bitter sesquiterpene lactones, coumarins, flavonoids and vitamins (Varotto et al, 2000). *Cichorium intybus* is a popular Ayurvedic remedy for the treatment of liver diseases. It is commonly known as Kasni. The plant root is used as antihepatotoxic, antiulcerogenic, antiinflammatory, appetizer, digestive, stomachic, liver tonic, cholagogue, cardiotonic, depurative, diuretic, emmenagogue, febrifuge, alexeteric and also as tonic. It is useful in vitiated conditions of kapha and pitta, cephalalgia, heapatomegaly, inflammations, anorexia, dyspepsia, flatulence, colic, gout, burning sensation, allergic conditions of skin, jaundice, splenomegaly, hyperdipsia, skin diseases, leprosy, strangury, amenorrhoea, chronic and bilious fevers, ophthalmia, pharyngitis, vomiting arthralgia, lumbago, asthma and general debility (Nadkarni, 1976 and Rastogi et al., 1994).

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High-performance liquid chromatography (HPLC) is a powerful analytical technique due to its merits of reliability, simplicity, reproducibility and speed (Alok et al., 2011). As part of our work, we report a simple, rapid and selective HPLC method for the separation and determination of Cichorin in crude plant material. The aim of this work was to develop an accurate, specific, repeatable and robust method for the determination of Cichorin. The proposed method was validated in compliance with International Conference on Harmonization (ICH) guidelines (ICH, 1997).

Material and Methods
Collection and identification of raw material
Dried leaves of *Cichorium intybus* were received from the Taxonomist, Dabur Research and Development Center, Sahibabad, Ghaziabad (U.P.). The plant material was identified by Dr. G.P.Kimothi, Taxonomist, Dabur Research and Development Center. A specimen has been retained in the department for future reference.

Chemicals and reagents
All the chemicals used in the experiments were of HPLC grade and analytical grade.
Water was purified using a Mili-Q Academic A10 water purification system (Millipore, France). Solvents used for mobile phase were filtered through membrane (0.45µm pore size) and degassed before use.

HPLC and chromatographic conditions
The analysis was performed using high-performance liquid chromatographic system (Shimadzu class LC) which consisted of a FCV-10 ACVP Pump, DGU-14A degasser, a thermostated CTO-10ASVP column oven compartment, an autosampler and a SPD-M10AVP Diode Array detector. HPLC systems SPD-M10 AVP and SIL-20 AC were used as equipments I and II for intermediate precision studies. A reverse phase Zorbax ODS II (250 mm × 4.6 mm, 5 µm) column was used. All analysis was performed at the column temperature of 40 ± 1 °C with a mobile phase of Methanol: Water (1: 1), an injection volume of 10 µL and a flow rate of 1.0 mL/min. The UV absorbance of eluent was measured at 346 nm.

Preparation of Stock solution
Accurately weighed 2.1 mg of Standard Cichorin was dissolved in 10 ml of methanol. Transferred 1ml of the resulting solution into a 25 ml volumetric flask, and volume made upto the mark with methanol.

Preparation of Test solution
Around 5.00g of drug was weighed and refluxed with methanol (100 ml) for 1 h over water bath and filtered

through Whatman filter paper (No. 41). The marc left out was refluxed again with 50 ml of methanol twice for 30 min each and filtered. The filterates were combined and concentrated to 50 ml in rotary vacuum evaporator and the resulting solution was used as test solution.

Method validation

Precision

Repeatability of the sample application and measurement of peak area were carried out using six replicates of the same sample and was expressed in terms of percent relative standard deviation (%RSD)

Recovery studies

The pre-analyzed samples were spiked with extra 80, 100 and 120% of the standard Cichorin and the mixtures were reanalyzed by the proposed method. The experiment was conducted six times. This was done to check for the recovery of the Cichorin at different levels in the plant material.

Specificity

The specificity of the method was ascertained by analyzing the standard drug and plant material. The peak for Cichorin in the sample was confirmed by comparing the retention times of the sample peak with that of the standard. The peak purity of the Cichorin was assessed by comparing the spectra at two levels, viz. peak start (S) and peak end (E) positions.

Results and discussion

In order to develop and validate an efficient method for the analysis of cichorin in crude raw material, we explored the detection wavelength, different solvent systems and the compositions of mobile phase. According to preliminary results, we finalized the detection wavelength of 346nm and the mobile phase of methanol: water (1:1) in an isocratic flow. Before fully implemented in the quantitative determination of cichorin, this method was thoroughly validated for its linearity, specificity, accuracy, precision and intermediate precision, robustness under various modified conditions.

Calibration curve and linearity

The calibration curve was generated from seven concentrations levels, i.e. 5.3, 6.11, 7.13, 7.96, 8.4, 9.04 and 10.5 µg/ml and the corresponding peak areas. It demonstrated an excellent linearity in the range of 5.3-10.5 µg/ml of cichorin. The linear equation for the calibration curve was y=23337x+22557 with a correlation coefficient of 0.991. Figure 1 displays the calibration curve for cichorin at 346 nm.

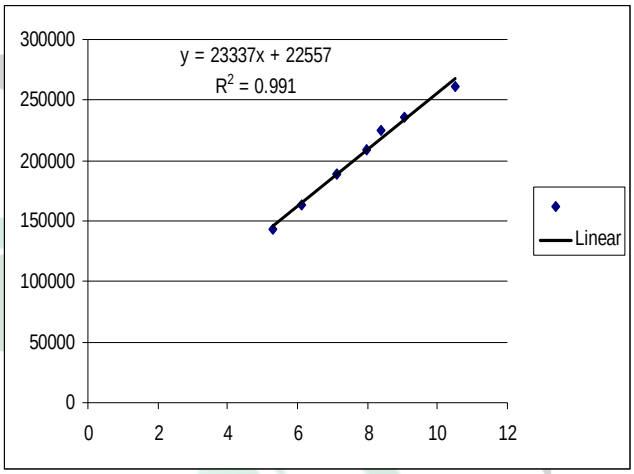
Precision

The %RSD for six replicate injections of the standard drug cichorin and measurement of peak areas was found to be 0.78%.

Six samples of a single batch of the crude herb powder were prepared and analyzed by the proposed method. The %RSD of 1.52% indicates that the method has an acceptable level of precision.

Recovery

The results showed high efficiency for extraction of cichorin from crude powder material. The recovery of cichorin ranged from 96.30%-105.6%. This confirms that the proposed method can be used for determination and quantification of Cichorin.



Graph 1: Calibration curve and linearity

The HPLC technique we have described is precise, specific and accurate for the determination of Cichorin. Statistical analysis proves that the method is reproducible and selective for the analysis of cichorin. Its advantages are speed and simplicity of sample treatment, satisfactory precision and accuracy.

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