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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ERGOTAMINE TARTRATE AND CAFFEINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A new method was established for simultaneous estimation of Ergotamine tartrate and Caffeine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Ergotamine tartrate and Caffeine by using Agilent C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer ph4.0 : ACN (30:70%v/v), detection wave length was 254nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2.The retention times were found to be 2.507 mins and 3.233 mins. The % purity of Ergotamine tartrate and Caffeine was found to be 100.3% and 101.1% respectively. The system suitability parameters for Ergotamine tartrate and Caffeine such as theoretical plates and tailing factor were found to be 1.3, 5824.4 and 1.2, 2936.0 the resolution was found to be 9.4.The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Ergotamine tartrate and Caffeine was found in concentration range of 20µg-100µg and 20µg-100µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 102.5% and 101.0%, %RSD for repeatability was 0.6 and 0.5, % RSD for intermediate precision was 0.7 and 0.6 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.1 and 3.02, and LOQ value was 10.1 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Ergotamine tartrate and Caffeine in API and Pharmaceutical dosage form.

Keywords :-Agilent C18, Ergotamine tartrate and Caffeine, RP-HPLC

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INTRODUCTION

The components of a basic High-Performance Liquid Chromatography [HPLC] system are shown in the simple diagram in figure 5. A reservoir holds the solvent [called the mobile phase, because it moves]. A high-pressure pump [solvent delivery system or solvent manager] is used to generate and meter a specified flow rate of mobile phase, typically millilitres per minute. An injector is able to introduce [inject] the sample into the continuously flowing mobile phase stream that carries the sample into the HPLC column.

The column contains the chromatographic packing material needed to effect the separation. This packing material is called the stationary phase because it is held in place by the column hardware. A detector is needed to see the separated compound bands as they elute from the HPLC column. The mobile phase exits the detector and can be sent to waste, or collected, as desired. When the mobile phase contains a separated compound band, HPLC provides the ability to collect this fraction of the elute containing that purified compound for further study. This is called preparative chromatography.

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The detector is wired to the computer data station, the HPLC system component that records the electrical signal needed to generate the chromatogram on its display and to identify and quantitative the concentration of the sample constituents. Since sample compound characteristics can be very different, several types of detectors have been developed. For example, if a compound can absorb Ultra Violet light, a UV-absorbance detector is used. If the compound does not have either of these characteristics, a more universal type of detector is used, such as an Evaporative-Light-Scattering Detector [ELSD]. The most powerful approach is the use multiple detectors in series. For example, a UV and/or ELSD detector may be used in combination with a Mass Spectrometer [MS] to analyze the results of the chromatographic separation. This provides, from a single injection, more comprehensive information about an analyte. The practice of coupling a mass spectrometer to an HPLC system is called LC/MS.
The most simplified way of explaining the cycle of operation, without taking into account the compressibility of the solvents, is as follows. From the moment when the outlet valve of cylinder a closes and its entrance valve open, the piston in A, moving backwards, sucks the eluent through the inlet check valve and the chamber fills. Meanwhile cylinder B is open and its piston moves forward to force the mobile phase towards the injector and the column. The volume displaced by piston B is half of that available in the chamber of piston A. With chamber A full, the entrance valve of a closes and the corresponding outlet valve opens. Piston a now advances and pushes out the contents of the chamber. Half of this volume is expelled directly towards the column, the other half serves to fill cylinder B as piston B retracts. A pulse absorber is located between the two cylinders (diagram courtesy of Agilent Technologies).

Method validation can be defined as per ICH "Establishing documented evidence which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics". Both caffeine citrate injection for intravenous administration and caffeine citrate oral solution are clear, colorless, sterile, non-pyrogenic, preservative-free, aqueous solutions adjusted to pH 4.7. Each mL contains 20 mg caffeine citrate (equivalent to 10 mg of caffeine base) prepared in solution by the addition of 10 mg caffeine anhydrous, USP to 5 mg citric acid monohydrate, USP, 8.3 mg sodium citrate dihydrate, USP and Water for Injection, USP.

Ergotamine tartrate, originally derived from a rye fungus, Clavicepspurpurea, is a serotonin receptor agonist, available in combination with caffeine as a tablet or suppository. Caffeine citrate, sold under the brand name Cafcit among others, is a medication used to treat a lack of breathing in premature babies. Specifically it is given to babies who are born at less than 35 weeks or weigh less than 2 kilograms once other causes are ruled out.

ICH Method validation parameters

For chromatographic methods used in analytical applications there is more consistency in validation. Related substances are commonly present in the pharmaceutical products but those are always within the limits as specified in ICH (Q2B).

- Specificity
- Linearity
- Accuracy
- Precision
- Limit of Detection
- Limit of Quantitation
- Robustness
- System suitability

MATERIALS AND METHODS

MATERIALS:

The list of instruments used in the course of experimental work is as follows:

Table 1: List of Instruments

S.No.	Instrument	Model No.	Software	Manufacturer's name	S.No.
1	HPLC Alliance PDA Detector	Waters 2695 Waters 996	Empower	Waters	1
2	UV double beam spectrophotometer	UV 3000	UV Win 5	Lab India	2
3	Digital weighing balance	BSA224SCW	-	Satorius	3
4	pH meter	AD102U	-	Lab India	4
5	Ultra sonicator	SE60US	-	-	5

The experimental work involves several chemicals. Chemicals used presently are listed below:

Table 2: List of Chemicals

S.No.	Chemical	Manufacturer	Grade
1	Water	Merck	HPLC Grade

2	Methanol	Merck	HPLC Grade
3	Acetonitrileeeee	Merck	HPLC Grade
4	Potassium dihydrogen orthophosphate	Merck	A.R
5	Ergotamine tartrate and Caffeine API	-	-

METHOD DEVELOPMENT:

Method development for simultaneous estimation of Ergotamine tartrate and Caffeine Pharmaceutical dosage forms includes the following steps:

1. Selection of detection wavelength (λmax)
2. Selection of column
3. Selection of mobile phase
4. Selection of flow rate
5. Preparations and procedures

1. Selection of Detection wavelength:

10 mg of Ergotamine tartrate and Caffeine was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overla y spectrum was used for selection of wavelength for Ergotamine tartrate and Caffeine. The isobestic point was taken as detection wavelength.

2. Selection of column:

Column is selected based on solubility, polarity and chemical differences among Analytes [Column: Agilent C18 (4.6 x 250mm, 5µm]

3. Selection of mobile phase:

Phosphate buffer : ACN (70:30%v/v) has been selected as mobile phase. If any buffer selected buffer pH should be between 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved. If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation. Good Response, Area, Tailing factor, Resolution will be achieved.

4. Selection of flow rate:

Flow rate selected was 1ml/min

Flow rate is selected based on

1. Retention time
2. Column back pressure
3. Peak symmetry
4. Separation of impurities

5. Preparations and procedures:

Preparation of mobile phase:

A mixture of Phosphate buffer pH 4.0 300ml (30%), 700 mL of ACN (30%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

Diluant Preparation:

Mobile phase is used as Diluant.

Preparation of the individual Ergotamine tartrate standard preparation:

10mg of Ergotamine tartrate working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of the individual Caffeine standard preparation:

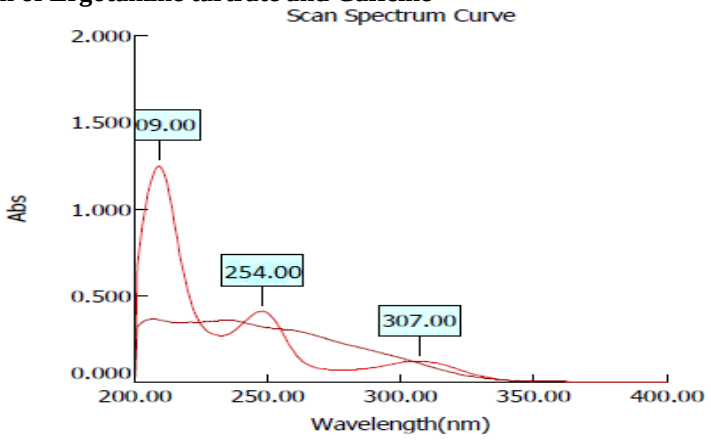
10mg of Caffeine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluent

RESULTS AND DISCUSSIONS

Wavelength Detection:

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Ergotamine tartrate and Caffeine was obtained and the isobestic point of Ergotamine tartrate and Caffeine showed absorbance's maxima at 254 nm.

Figure 1: Overlay spectrum of Ergotamine tartrate and Caffeine



METHOD DEVELOPMENT

The chromatographic method development for the simultaneous estimation of Ergotamine tartrate and Caffeine were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the optimized chromatographic method was selected for the separation and quantification of Ergotamine tartrate and Caffeine in API and pharmaceutical dosage form by RP-HPLC method.Ergotamine tartrate and Caffeine. Each three injections of sample and standard was inject into chromatographic system.

Figure 2: Chromatogram showing sample injection-1

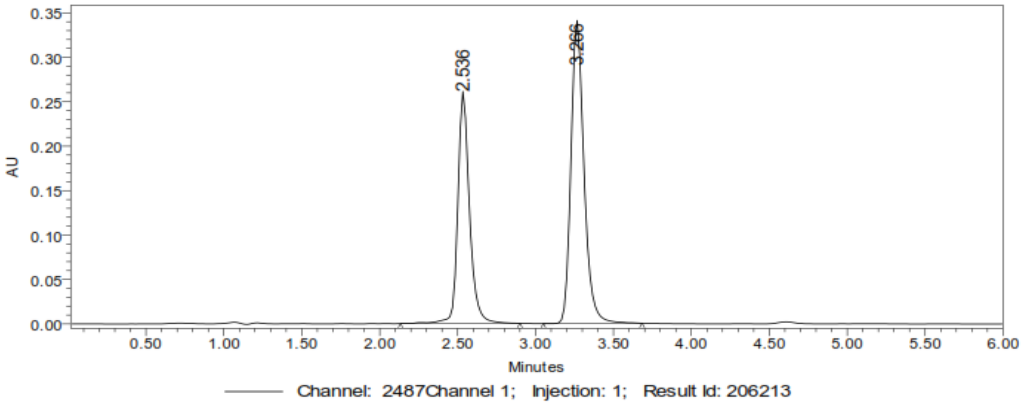


Figure 3: Chromatogram showing sample injection-2

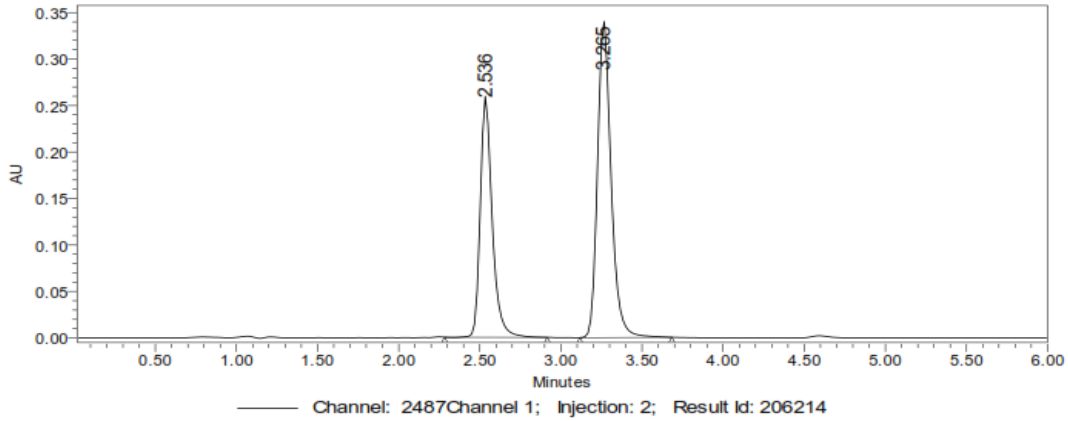


Figure 4: Chromatogram showing standard injection-1

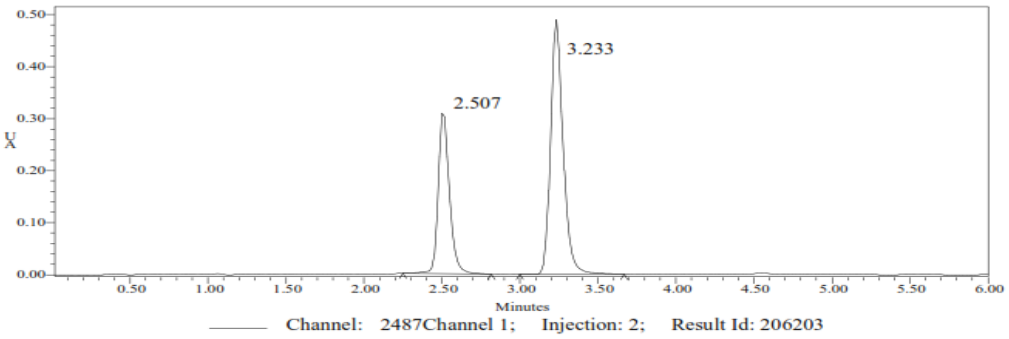
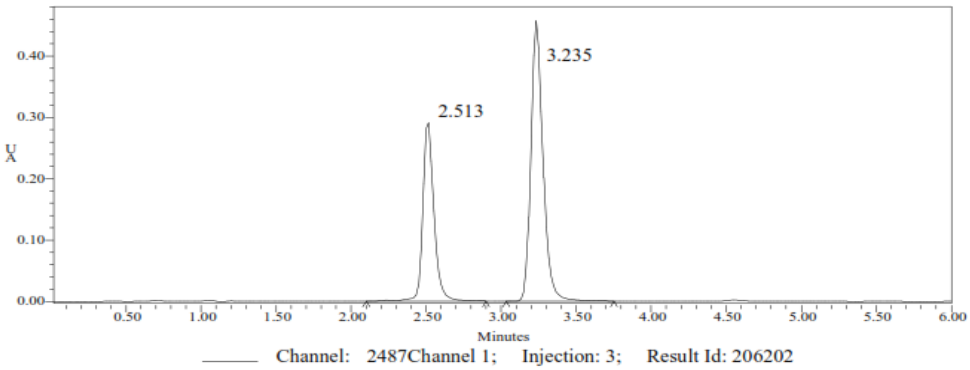


Figure 5: Chromatogram showing standard injection-2



Assay Calculations for Ergotamine Tartrate and Caffeine

The assay study was performed for thevalidation of Caffeine

VALIDATION RESULTS

Accuracy:The accuracy study was performed for 50%, 100% and 150 % for Ergotamine tartrate and Caffeine.Each level was injected in triplicate into chromatographic system.The area of each level was used for calculation of % recovery.

The accuracy results for Ergotamine tartrate

Table 3: Accuracy results of Ergotamine tartrate

%Concentration (at specification Level)	Area	Amount added(m)	Amount found(m)	% Recovey	Mean Recovery
50%	1426646	5	4.9	101.8%	102.5%
100%	2551005	10	9.98	99.9%	
150%	2139845	15	15.0	100.0%	

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%.

The accuracy results for Caffeine

Table 4: Accuracy results of Caffeine

%Concentration(at specification level)	**Area**	**Amount Added(mg)**	**Amount Found(mg)**	**% Recovery**	**Mean Recovery**
50%	975578	5	5.0	101.3%	
100%	1718370	10	9.96	99.6%	101.0%
150%	1465857	15	14.9	99.3%	

Acceptance criteria:

The % recovery for each level should be between 98.0 to 102.0 %.

Precision

i) Repeatability

ii) Intermediate precision (Ruggedness)

Repeatability

The precision study was performed for five injections of Ergotamine tartrate and Caffeine. Each standard injection was injected in to chromatographic system. The area of each Standard injection was used for calculation of % RSD.

Table 5: Repeatability results of Ergotamine tartrate and Caffeine

Name: Ergotamine tartrate

	Name	RT	Area	Height (µV)	USP Plate Count	USP Tailing
1	Ergotamine	2.506	1553631	316525	6346.5	1.3
2	Ergotamine	2.516	1508002	296974	6197.1	1.2
3	Ergotamine	2.519	1545624	307327	6184.0	1.3
4	Ergotamine	2.531	1542374	302327	6176.0	1.2
5	Ergotamine	2.544	1561368	302525	6382.1	1.3
Mean			1542200			
Std. Dev.			20490.0			
% RSD			1.33			

Name: Caffeine

	Name	RT	Area	Height (µV)	USP Plate Count	USP Tailing
1	Caffeine	3.230	2790868	497608	7950.1	1.2
2	Caffeine	3.239	2661482	468477	8046.5	1.2
3	Caffeine	3.246	2706096	474632	8054.1	1.2
4	Caffeine	3.257	2703419	473234	8171.8	1.2
5	Caffeine	3.271	2695932	474830	8068.3	1.2
Mean			2711560			
Std. Dev.			47796.3			
% RSD			1.76			

Intermediate precision/Ruggedness

The intermediate precision study was performed for five injections of Ergotamine tartrate and Caffeine. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of % RSD.

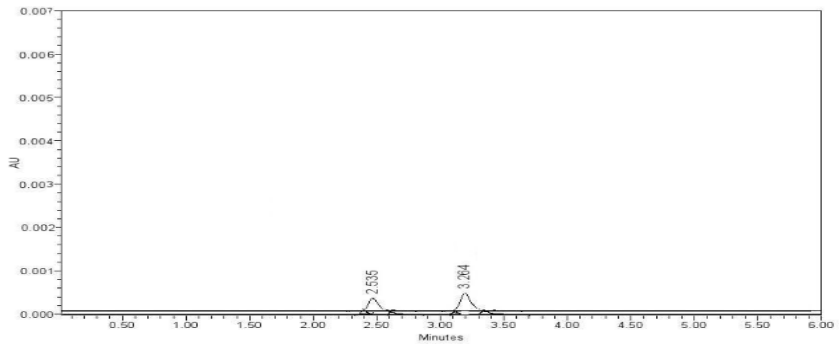
Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank.

Detection of limit:

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines

Figure 6: Results of LOD



Qantitation Limit:

Caffeine

Calculation of S/N Ratio	:	Average
Baseline Noise obtained from Blank	:	42 µV
Signal Obtained from LOQ solution	:	422µV

S/N = 422/42 = 10.0

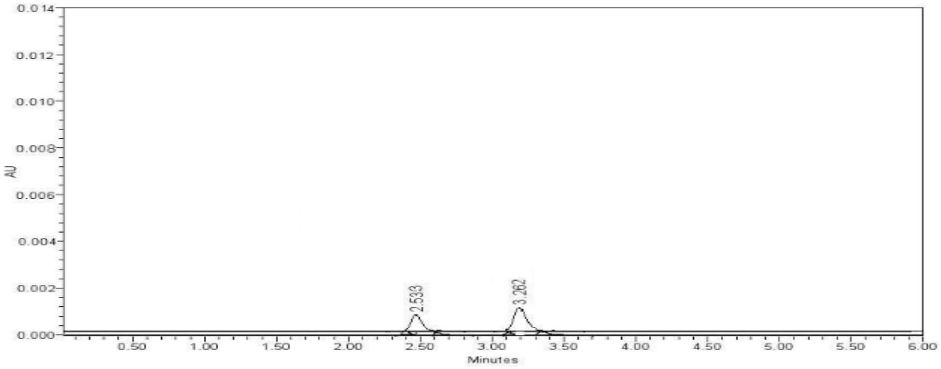
Acceptance Criteria	:	S/N Ratio value shall be 10 for LOQ solution.

Ergotamine tartrate

Calculation of S/N Ratio	:	Average
Baseline Noise obtained from Blank	:	40 µV
Signal Obtained from LOQ solution	:	404µV

S/N = 404/40 = 10.1

Figure 7: Results of LOQ



Linearity:

The linearity study was performed for the concentration of 100ppm to 500ppm and1ppm to 5ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient.

Table 6: Linearity Results Caffeine

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	471543
2	II	40 ppm	656277
3	III	60 ppm	794999

4	IV	80 ppm	946124
5	V	100 ppm	1002139
Correlation Coefficient			0.999

Table 7: Linearity Results Ergotamine tartrate

S.No	Linearity Level	Concentration	Area
1	I	20 ppm	471543
2	II	40 ppm	656277
3	III	60 ppm	794999
4	IV	80 ppm	946124
5	V	100 ppm	1002139
Correlation Coefficient			0.999

Figure 8: Calibration curve of Caffeine

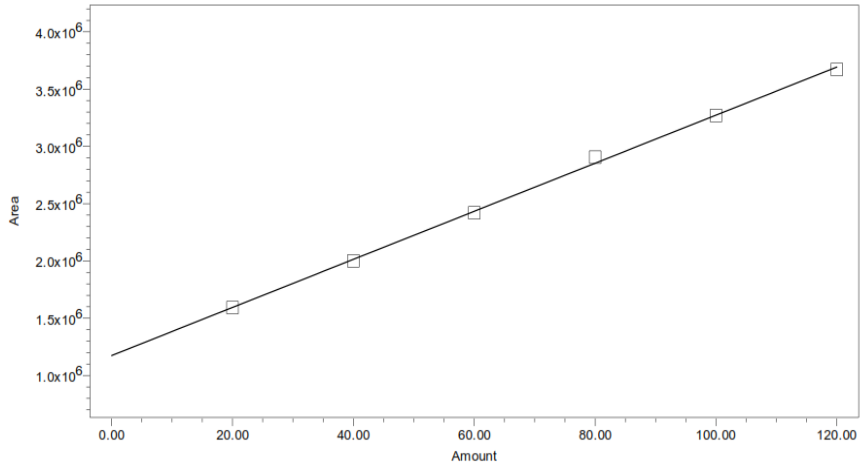
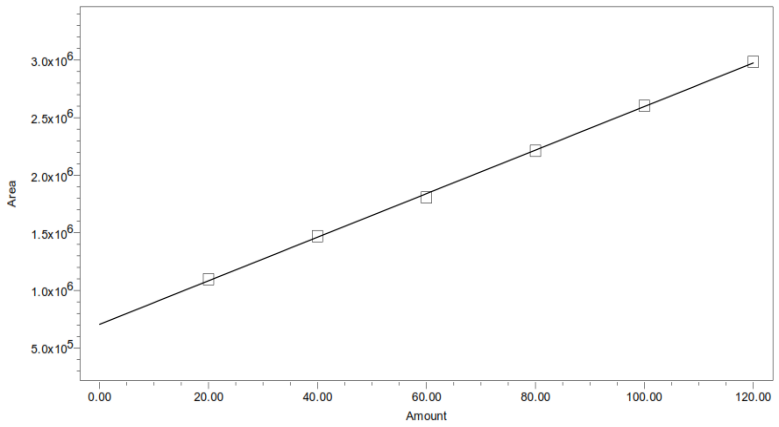


Figure 9: Calibration curve of Ergotamine tartrate



Range

The linearity study was performed for concentration range of 20µg - 100µg and20µg-100µg of Ergotamine tartrate and Caffeine and the correlation coefficient was found to be 0.999 and 0.999. (NLT 0.999).

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

CONCLUSION

A new method was established for simultaneous estimation of Ergotamine tartrate and Caffeine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Ergotamine tartrate and Caffeine by using Agilent C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer ph4.0 : ACN (30:70%v/v), detection wave length was 254nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The retention times were found to be 2.507 mins and 3.233 mins. The % purity of Ergotamine tartrate and Caffeine was found to be 100.3% and 101.1% respectively. The system suitability parameters for Ergotamine tartrate and Caffeine such as theoretical plates and tailing factor were found to be 1.3, 5824.4 and 1.2, 2936.0 the resolution was found to be 9.4.The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Ergotamine tartrate and Caffeine was found in concentration range of 20µg-100µg and 20µg-100µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 102.5% and 101.0%, %RSD for repeatability was 0.6 and 0.5, % RSD for intermediate precision was 0.7 and 0.6 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.1 and 3.02, and LOQ value was 10.1 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Ergotamine tartrate and Caffeine in API and Pharmaceutical dosage form.

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