بسم الله الرحمن الرحيم

Sudan University of Science and Technology

Collage of Graduate studies

Development and Validation of some Analytical Methods for Quantitative Determination of hyoscine butyl bromide

التطوير و التحقق لبعض الطرق التحليلية للتقدير الكمي للهيوسين بيوتيل بروميد

By

Shaza Mamoon Ibrahim Alseddig

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Supervisor:

Prof.Dr.Ahmed Elsadig Mohammed Saeed

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الآية

بسم الله الرحمن الرحيم

﴿ اللَّهُ لَا إِلَهَ إِلَّا هُوَ الْحَيُّ الْقَيُّومُ لَا تَأْخُذُهُ سِنَةٌ وَلَا نَوْمٌ لَهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الْأَرْضِ مَنْ ذَا الَّذِي يَشْفَعُ عِنْدَهُ إِلَّا بِإِذْنِهِ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ وَلَا يُحِيطُونَ بِشَيْءٍ مِنْ عِلْمِهِ إِلَّا بِمَا شَاءَ وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالْأَرْضَ وَلَا يَئُودُهُ حِفْظُهُمَا وَهُوَ الْعَلِيُّ الْعَظِيمُ ﴾

صدق الله العظيم

(سورة البقرة _ الأية 255)

Dedication

I dedicated thispieceof work with deep love and respect to

my mother

A strong and gently soul who taught me to trust in Allah, believe that every challenge needs self efforts and hard work as well as guidance of elders epically those who were very close to our heart .

my father

who I could never done this without his faith, support, and encouragement. thank you for teaching me to believe in god ,in myself , and in my dreams .

It's also dedicated to my brother , my sister and my lovely friends who supporting me during my whole life.

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Thanks to Allah the most gracious, Merciful for giving me the strength, health and patience to accomplish this research for a master's degree in chemistry.

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I would like to thank the UNAMID pharmaceutical factory ,AZAL for Pharmaceutical Industries and CLIMAX pharmaceutical factory , for good cooperation because they are available willingly to provide assistance when requested, and I'm grateful for their employees in the chemistry lab to help them.

Finally, a special thanks to all those who helped me morally.

Abstract:

Method validation is the process used to confirm that the analytical procedure employed for specific test is suitable for its intended use. Result from method validation can be used to judge the quality, reliability and consistency of analytical results; it is integral part of any good analytical practice. When extended to analytical procedure depending upon the application, it means that a method work reproducibly, when carried out by same or different persons, in same or different laboratories , using different chemicals or reagents and using different equipments at different condition or environment.

The aim of the this study was to develop and validate HPLC method and UV Spectrophotometeric method for the determination of hyoscine butyl bromide. In RP- HPLC a mixture 1.0 g of sodium dodecyl sulfate in a mixture of 370 mL of 0.001M hydrochloric acid and 750 mL of methanol (in 1000 ml) (v/v) was used as a modified mobile phase mixture. the column was C8 (25×4.6mm). The flow rate was set to 2ml/mint. The method was validated regarding linearity, range, limit of detection, limit of quantitation, precision, and accuracy.

The obtained results showed that the validated method have good linearity, accuracy, precision and selectivity and reducing the retention time of hyoscine butyl bromide from (14.26) minutes to (9.0) minutes. Analytical method development results indicated thatthe limit of detection was (0.0054µg/ml), limit of quantitation was (0.0165µg/ml)

and assay exhibited a linear over the range of (10-50µg/ml). The modified method of chromatography gave a linear relationship at a wavelength of 210 nm, linear correlation coefficient (0.998).

In the UV, The method validated according to international conference on harmonization guideline, linearity range, and limit of detection, limit of quantitation, precision, and accuracy. The modified method of UV gave a linear relationship at a wavelength of 257 nm, linear correlation coefficient (0.9999).The validated method showed results the indicated limit of detection was (0.0139µg/ml), limit of quantitation was (0.0423µg/ml).

Respectively results Were compared with the official method were conducted analyzes in two different days to determine relative standard deviation which did not exceed 2%, the developed methods in present research successfully in the quantitation analysis of commercial preparation tablets of different sources. The study summarized the results of the analysis are not different from those obtained by official method approved therefore developed method considered to be fit, the best because fast, inexpensive and more safe, which can be of use for routine analysis drug lab.

الخلاصة:

كان الهدف الأساسي من هذه الدراسة هوا لتطوير و التحقق من صحة الطريقة هي العملية المستخدمة للتأكد من دقة الإجراء التحليلي المستخدمة لاختبار معين هو مناسب للاستخدام المقصود. وذلكلاستخدام نتيجة التحقق المتحصل عليها للتأكد من صحة الطريقة للحكم على جودة ودقة النتائج التحليلية؛. عندتنفذيها من قبلنفس الشخص او أشخاص مختلفين ، في نفس أو مختبرات مختلفة، وذلك باستخدام مواد كيميائية مختلفة أو الكواشف واستخدام معدات مختلفة في بيئات مختلفة.

وكان هدف هذه الرسالة لتطوير والتحقق من صحة طريقة كروتوغرافيا السائل عالي الأداء ومطيافية الأشعة فوق البنفسجية لتحديد هيوسين بيوتيل بروميد. في كروتوغرافيا السائل عالي الأداء، تم تعديل الطريقة بتغير الطور المتحرك و ذلك باستخدام خليط 1.0 غرام من كبريتات دوديكيل الصوديوم في خليط من (370 مل من 0.001 مولارية من حمض الهيدروكلوريك و 750 مل من الميثانول (في 1000 مل) (v / v) كخليط للطور المتحرك باستخدام عمود فصل C8 بالأبعاد (4.6mm × 25). تم تعيين معدل التدفق إلى 2 مل / دقيقة . و أظهرت النتائج أن الطريقة دقيقة و انتقائية و ذات علاقة خطية حيث تم تقليل زمن الفصل من (14.26 دقيقة) إلى (9 دقائق) و كانت نتيجة حد الكشف (0.0054 ميكروغرام/مل) و حد الكشف الكمي (0.0165 ميكروغرام/مل)، كما أن الطريقة المعدلة أعطت علاقة خطية بمعامل ارتباط مقداره (0.998) عند طول موجي 210 نانو متر، تم التحقق من صحة الطريقة فيما يتعلق بالخطي، المدى، الحد من الكشف، الحد من الكميات والدقة.

في طريقة مطيافية الأشعة فوق البنفسجية تم التحقق من صحة الطريقة وفقا للمؤتمرات الدولية حول المبادئ التوجيهية للمواءمة، ونطاق الخطي، والحد من الكشف، والحد من الكميات والدقة. وقد أظهرت الطريقة المعدلة للأشعة فوق البنفسجية علاقة خطية عند طول موجة 257 نانومتر، بمعامل ارتباط خطي (0.9999) .وأظهرت الطريقة نتائج لحدالكشف بقيمة (0.0139ميكرةغرام / مل)، و حد التقدير الكمي(0.0423ميكروغرام / مل) النتائج على التوالي تم مقارنتها مع الطريقة الرسمية لتحديد الانحراف المعياري النسبي الذي لم يتجاوز 2٪.

و بذلك نجد أن الدراسة أثبتت أن نتائج التحليل لا تختلف عن تلك التي تم الحصول عليها بالطريقة الرسمية المعتمدة وان الطرق المحدثة تعتبر أفضل و ذلك لأنها سريعة وغير مكلفة وأكثر أمانا، و يمكن أن تكون ذات فائدة في معامل التحليل اليومية للأدوية.

List of content

List of tables

List of Figures

List of Abbreviations

BP: British pharmacopeia

USP: United State Pharmacopeia

ICH: International Conference on Harmonization

FDA: Food and Drug Administration

GMP: Good Manufacturing Practice

WHO: World Health Organization

HPLC: High Performance Liquid Chromatography

UV: Ultra Violet

ISO: International Standard Organization

QC: Quality Control

Mf. Date: Manufacture Date

EXP Date: Expire Date

B.NO: Batch Number

RSD: Relative Standard Deviation

SD: Standard Deviation

LOD: Limit of Detection

LOQ: Limit of Quantitation

Chapter One

1.INTRODUCTION

1.1Definition of validation

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications**."**(USP, 2007).

Validation of an analytical procedure is performed in order to demonstrate that the procedure is suitable for its intended use. Validation is performed in order to show that the result(s) generated by a particular analytical procedure are reliable and accurate;It is also a process that determines the fitness of an assay, which has been properly developed, optimized and standardized, for an intended purpose. All diagnostic assays should be validated for the species in which they will be used. An assay that has completed the first three stages of the validation pathway, including performance characterization, can be designated as "validated for the original intended purpose".

The objective of any analytical measurement is to obtain consistent, reliable and accurate data, which play a major role in achieving this goal. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice.The purpose is also to demonstrate that the procedure, when correctly applied, produces results that are fit for purpose. These guidelines describe the procedures to be carried out to validate the analytical procedures included as part of an application for approval of an active constituent and registration of an agricultural and veterinary chemical product, including those used in storage stability studies. They are not intended to apply to analytical methods for residue

analysis, biological and biotechnological products. Approaches other than those set forth in this guideline may be acceptable provided they are supported by adequate scientific justification**.**

1.2The Principle & guidelines of Validation

1.2.1ICH Guidelines

The objective of ICH is to increase international harmonization of technical requirements to ensure that safe, effective, and high quality medicines are developed and registered in the most efficient and cost effective manner.(ICH ,2007)

First of all, the ICH guidelines should be regarded as the basis and philosophical background to analytical validation, not as a checklist. “It is the responsibility of the applicant to choose the validation procedure and protocol most suitable for their product”. (Ermer and Miller,2005)

These guidelines focus mainly on the overall concept of validation and are intended as a basic guide for use by GMP inspectors and manufacturers. It is not the intention to be prescriptive in specific validation requirements, which includes the identification of the performance parameters relevant for the given procedure, the definition of appropriate acceptance criteria and the appropriate design of the validation studies.

ICH has developed over 45 harmonized guidelines; topics are divided into four major categories:

1_Quality (Q), i.e., those relating to chemical and pharmaceutical Quality Assurance

2_Safety (S), i.e., those relating to in vitro and in vivo preclinical studies

3_ Efficacy (E), i.e., those relating to clinical studies inhuman subject

4_Multidisciplinary topics (M), i.e., cross-cutting Topics which do not fit uniquely into one of the above categories

These guidelines serves as general guidance only, and the principles may be considered useful in its application in the manufacture and control of active pharmaceutical ingredients (APIs) and finished pharmaceutical products, and its aim to give guidance to inspectors of pharmaceutical manufacturing facilities and manufacturers of pharmaceutical products on the requirements for validation.(ICH , 2007)

1.2.2FDA Guidelines

According to FDA guidelines on general principle of process validation, process validation defined as establishing documented evidence, which provide a high degree of assurance, that specific process will consistently produce a product meeting its predetermined specification and quality characteristics.Two guidelines on validation were issued by the US Food and Drug Administration (FDA), the first one is intended to ensure that the analytical procedure can be applied in an FDA laboratory and therefore requires a detailed description of the procedure, reference materials, as well as a discussion of the potential impurities, etc. The secondguideline focuses on reversed-phase chromatography and provides a lot of details with regard to critical methodological issues, as well as some indication of acceptability of results.(Ajay, 2012)

1.2.3WHO Guidelines

The World Health Organization (WHO) published a guideline under the title, ‘Validation of analytical procedures used in the examination of pharmaceutical materials’. It appeared in the 32nd report of the WHO expert committee on ‘specifications for pharmaceutical preparations’

which was published in 1992.Also, in WHO GMP under the element "Qualification and Validation ", Validation of analytical test method, automated system and cleaning procedure has been emphasized. It's read as under "it's of critical importance that particular attention is paid to validation of analytical test method, automated system and cleaning procedure".(Pharvi,2010)

1.2.4GMP Guidelines

Process validation is requirement of current Good Manufacturing Practice (GMPs) for finished pharmaceutical and GMP regulation for medical devices and therefore applies to the manufacture of both drug product and medical device.

Validation is an essential part of good manufacturing practices (GMP). It is, therefore, an element of the quality assurance programme associated with a particular product or process. The basic principles of quality assurance have as their goal the production of products that are fit for their intended use. These principles are as follows:

• Quality, safety and efficacy must be designed and built into the product.
• Quality cannot be inspected or tested into the product.
• Each critical step of the manufacturing process must be validated.

Other steps in the process must be under control to maximize the probability that the finished product consistently and predictably meets all quality and design specifications.

Validation of processes and systems is fundamental to achieving these goals. It is by design and validation that a manufacturer can establish confidence that the manufactured products will consistently meet their product specifications.

Documentation associated with validation includes:-

- Standard operating procedures (SOPs)
- Specifications
- Validation master plan (VMP)
- Qualification protocols and reports
- Validation protocols and reports.

The implementation of validation work requires considerable resources such as:

• Time*:* generally validation work is subject to rigorous time schedules.

• Financial*:* validation often requires the time of specialized personnel and expensive technology.

• Human*:* validation requires the collaboration of experts from various disciplines (e.g. a multidisciplinary team, comprising quality assurance, engineering, manufacturing and other disciplines, depending on the product and process to be validated).

1.3Types of analytical procedures to be validated

Discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:-

1. Identification tests.

2. Quantitative tests: for impurities 'content'.

3. Limit tests for the control of impurities.

4. Quantitative tests of the active in samples of drug substance or drug product or other selected components in the drug product.

Different validation characteristics are required for a quantitative test than for a limit test.(ICH,1994)

1.4The criteria and parameters of validation

The scope of the method and its validation criteria should be defined. These include: compounds, matrices, type of information, qualitative or quantitative, detection and quantitation limits, linear range, precision and accuracy, type of equipment and location, and it should include the different types of equipment and the locations where the method will be run. The method's performance characteristics should be based on the intended use of the method. For example, if the method will be used for qualitative trace level analysis, there is no need to test and validate the method's linearity over the full dynamic range of the equipment. Initial parameters should be chosen according to the analyst's best judgment. Finally, parameters should be agreed between the lab generating the data and the client using the data.

In pharmaceutical industry the most comprehensive document was published as the quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures.

The various validation parameters are:

- Accuracy
- Precision (repeatability and reproducibility)
- Linearity and range
- Limit of detection (LOD)/ limit of quantitation (LOQ)
- Selectivity/ specificity
- Robustness/ ruggedness
- Stability and system suitability studies

It is important for one to understand the parameters or characteristics involved in the validation process.(Samuels,2009)

The various performance parameters, which are addressed in a validation exercise, are grouped as follows:

1.4.1Accuracy

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value found either the value accepted as a conventional true valueor an accepted reference valueIt is the measure of the exactness of the analytical method developed. The accuracy of an analytical method may be determined by any of the following ways:

1- Analyzing a sample of known concentration and comparing the measured value to the 'true' value. However, a well characterized sample (e.g., reference standard) must be used.

2- Spiked – placebo (product matrix) recovery method. In this method, a known amount of pure active constituent is added to formulation blank [sample that contains all other ingredients except the active(s)], the resulting mixture is assayed, and the results obtained are compared with the expected result.

3- Standard addition method. In this method, a sample is assayed, a known amount of pure active constituent is added, and the sample is again assayed. The difference between the results of the two assays is compared with the expected answer.

The accuracy of a method may vary across the range of possible assay values and therefore must be determined at several different fortification

levels. The accuracy should cover at least 3 concentrations (80, 100 and 120%) in the expected range.(Samuels,2009)

1.4.2Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. This is usually expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditionsas the standard deviation or the relative standard deviation (coefficient of variation).

Precision is a measure of the degree of reproducibility or of the repeatability of the analytical method under normal operating circumstances.

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at different occasions, different laboratories and different batch of reagent, different analysts and different equipment.It is normally expected that at least six replicates be carried out and a table showing each individual result provided from which the mean, standard deviation and co-efficient of variation should be calculated for set of n value. The RSD values are important for showing degree of variation expected when the analytical procedure is repeated several time in a standard situation. Table showed RSD below 1% for built drugs and RSD below 2% for assays in finished product.(Samuels,2009)

Table (1.1): shown precision range of component of sample

Component measured in sample	Precision
≥ 10.0%	≤ 2.0%
1.0 Up to 10.0%	≤ 5%
0.1 Up to 1.0%	≤ 10%
< 0.1%	≤ 20%

1.4.3 Linearity

The linearity of an analytical method is its ability to elicit test results that are directly (or by a well-defined mathematical transformation) proportional to the analyte concentration in samples within a given range, itis usually expressed in terms of the variance around the slope of regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte.

The linear range of detect ability that obeys Beer's law is dependent on the compound analyzed and the detector used. The working sample concentration and samples tested for accuracy should be in the linear range.

The claim that the method is linear is to be justified with additional mention of zero intercept by processing data by linear least square regression, the test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method

of least squares.(correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be submitted for minimum of 5 concentrations is recommended)

Data is processed by linear least square regression declaring the regression co-efficient and b of the linear equationtogether with the correlation coefficient of determination (r). For the method to be linear the r value should be close to ±1.

$y = ax + b$ ---------- (1)

(Samuels,2009)

1.4.4Range

The range of an analytical method is the interval between the upper and lower levels of the analyte (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written.

The following minimum specified ranges should be considered:-

• For the assay of an active substance or a finished product: normally from 80 to 120 percent of the test concentration. If assay and purity are performed together as one test and only a 100% standard is used, linearity should cover the range from the reporting level of the impurities to 120 percent of the assay specification.

• For content uniformity, covering a minimum of 70 to 130 percent of the test concentration, unless a wider, more appropriate range, based on the nature of the dosage form (e.g., metered dose inhalers), is justified.

• For dissolution testing: ± 20 percent over the specified range. e.g., if the specifications for a controlled released product cover a region from 20%, after 1 h, up to 90%, after 24 h, the validated range would be 0-110% of the label claim.(Samuels,2009)

1.4.5 Limit of Detection

The limit of detection (LOD) of an analytical procedure is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantities. It is a limit that specifies whether or not an analyte is above or below certain value.

The LOD of detection of instrumental procedures is carried out by determining the signal-to noise ratio by comparing test results from the samples with known concentration of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected.

A signal-to-noise ratio of 2:1 or 3:1 is generally accepted. The signal-to noise ratio is determined by dividing the base peak by the standard deviation of all data points below a set threshold. Limit of detection is calculated by taking the concentration of the peak of interest divided by three times the signal-to-noise ratio.

The detection limit (DL) may be expressed as:

$DL = 3.3\ \sigma/S$ ---------- (2)

- **σ** = the standard deviation of the response
- **S** = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

The estimate of σ may be carried out in a variety of ways, for example:

- Based on the Standard Deviation of the Blank
- Based on the Calibration Curve.

(Samuels,2009)

1.4.6 Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy, and a parameter of quantitative assays for low levels of compounds in sample matrices, which is used particularly for the determination of impurities and/or degradation products.

A signal-to noise ratio of 10 to 1 is used to determine LOQ, which is measured by analyzing samples containing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision.

Quantitation limit (QL) may be expressed as:

$QL = 10\ \sigma/S$ ---------- (3)

σ = the standard deviation of the response

S = the slope of the calibration curve

The estimate of σ may be carried out in a variety of ways, for example:

- Based on the Standard Deviation of the Blank
- Based on the Calibration Curve

Where, the final assessment is based on an instrumental reading, the magnitude of background response by analyzing a number of blank samples and calculating the standard deviation of this response.(Samuels,2009)

1.4.7Selectivity (Specificity)

Selectivity of a method refers to the extent to which it can determine particular analyte(s) in a complex mixture without interference from other components in the mixture. The terms selectivity and specificity have often been used interchangeably. The term specific generally refers to a method that produces a response for a single analyte only, while the term selective refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other. If the response is distinguished from all other responses, the method is said to be selective. Since very few analytical methods respond to only one analyte, the use of the term selectivity is more appropriate than specificity.

The International Union of Pure and Applied Chemistry (IUPAC) have expressed the view that "Specificity is the ultimate of Selectivity'. The IUPAC discourages use of the term specificity and instead encourages the use of the term selectivity.

The selectivity of the analytical method must be demonstrated by providing data to show the absence of interference peaks with regard to degradation products, synthetic impurities and the matrix (excipients present in the formulated product at their expected levels).

The selectivity of chromatographic methods may be assessed by examination of peak homogeneity or peak purity test (e.g., diode array, mass spectrometry) to show that the analyte chromatographic peak is not attributable to more than one component.

Identification*:* To ensure the identity of an analyte.

Purity Tests*:* To ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of ananalyte, i.e. related substances test, heavy metals, residual solvents content, etc.

Assay (content or potency): To provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.(Pharvi,2010)

Table (1.2) Method validation acceptance criteria

Validation element	**Recommendation**	**Acceptance Criteria**
Linearity and rang	Minimum 5 points Slope Y Intercept Correlation coefficient	RSD≤2 Y close to zero R2 ≥ 0.99
Precision and repeatability	6 replicate analysis of sample at 100% test condition	RSD ≤2
Accuracy	Triplicate spiking of placebo at 80, 100,120% of label calim	98-102%
Intermediate precision	6 replicate analysis by different analyst on different day	RSD ≤2

Table(1.3): Recommended Validation characteristics of various Types of Tests

Type of analytical procedure **Characteristics**	**IDENTIFICATION**	**TESTING FOR IMPURITIES**		**ASSAY** **-dissolution (measurement only)** **-content/potency**
		Quantitative	**Limit**	
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Interm .Precision	-	+(1)	-	+(1)
Specificity	+	+	+	+
Detection limit	-	-(3)	+	-
Quantitation limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this characteristic is not normally evaluated

+ signifies that this characteristic is normally evaluated

(BP,2012)

1.5 Hyoscine Butyl Bromide

Hyoscine-N-Butyl bromide (HBB) is a derivative of hyoscine which is extracted from the leaves of the Duboisia tree found mainly in Australia, which is also known as Scopolamine-N-butyl bromide, N-Butyl ScopolammoniumBromide, and butyl scopolamine.Itis a quaternary ammonium compound which blocks the action of acetylcholine at parasympathetic sites (both muscarinic and nicotinic receptors) in smooth muscle, and in secretory glands. It causes decreased motilityof the gastrointestinal tract and the urogenital tracts, and is useful in the

treatment of spasms in these regions.It may also be useful in certain procedures, such as colonoscopy and sigmoidoscopy, and may be useful in the management of renal colic (although NSAIDsseem clinically superior). The role of HBB in the management of esophageal food obstruction is unclear at this time; further studiesneed to be done. (Samuels,2009)

1.5.1 General Structure

Trade Name : Buscopan

Chemical Name: Hyoscine butylbromide

Molecular formula :

$C_{21}H_{30}BrNO_4$

Molar mass :440.4

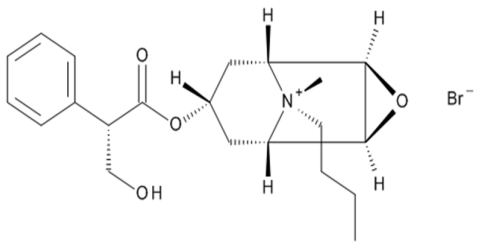


Figure (1.1): Hyoscine Butyl bromide General Structure

1.5.2 General Properties

A white or almost white, crystalline powder., almost odorless powder. The melting point is 139 °C to 141 °C.. The molecular weight is 440.4**.** Freely soluble in water and in methylene chloride, sparingly soluble in anhydrous ethanol.

1.5.3 Mechanism of Action

Hyoscine competitively blocks muscarinic receptors and has central and peripheral actions. It relaxes smooth muscle and reduces gastric and intestinal motility.(Saulo,2014)

Hyoscine butyl bromide blocks the action of acetylcholine at parasympathetic sites in smooth muscle, and secretory glands. Both its primary therapeutic effects and its side effects are based on this. Thus:

1. It causes decreased motility of the gastrointestinal tract and the urogenital tracts, and is useful in the treatment of spasms in these regions, as may be seen in gastroenteritis, colitis, inflammatory bowel disease, diverticulitis, biliary colic, cystitis, ureteric colic, and primary dysmenorrhea. It is also used to prevent spasms of the gastrointestinal tract prior to invasive radiologic and diagnostic procedures, such as endoscopic retrograde cholangiopancreatography (ERCP) and colonoscopy.

2. Its inhibitory action on glands in the oral cavity, gastrointestinal tract, and respiratory tract causes a reduction in their activity, with a consequent reduction in secretions. This makes the drug useful prior to induction of general anesthesia, and as a part of adjuvant management in cases of intestinal obstruction, in circumstances where surgical correction is not feasible (e.g. peritoneal carcinomatosis).

(Samuels ,2009)

1.5.4 PHARMACOLOGY

Butyl scopolamine is used to treat menstrual cramps or other spasmodic activity in the digestive system. It is not an analgesic in the normal sense,

since it doesn't 'mask' or 'cover over' the pain, but rather works to prevent painful cramps and spasms from occurring in the first place.

Hyoscinebutylbromide is a peripherally acting antimuscarinic, ant cholinergic agent used as an abdominal-specific antispasmodic. It is a quaternary ammonium compound and a semisynthetic derivative of scopolamine. It is also used to treat pain and discomfort caused by abdominal cramps, menstrual cramps, or other spasmodic activity in the digestive system. It is also effective at preventing bladder spasms. It is not an analgesic in the normal sense, since it doesn't 'mask' or 'cover over' the pain, but rather works to prevent painful cramps and spasms from occurring in the first place.

Hyoscine butyl bromide is an antispasmodic medicine which is taken to relieve cramps in the stomach, intestines or bladder. In particular, it helps to ease bloating and the spasm-type pain that can be associated with irritable bowel syndrome and diverticular disease. And it is known as an ant cholinergic medicine, it relieves the pain of cramps by helping your digestive system to relax.(Saulo,2014)

In summary, hyoscinebutylbromide appears to be a valuable treatment option for patients with symptoms of abdominal pain or discomfort associated with cramping.

1.5.5 Side effect

Like all medicines, hyoscine butylbromide can cause side effects although not everybody will experience them. Some of the more common side effects include blurred vision, a dry mouth, dizziness, increased heart rate, constipation and pain at the injection site. If you have any concerns about side effects, please speak to the staff caring foryou.(Guy’s and St Thomas’, 2014)

1.6 Aim & Objective

The aim of this study is to developed and validate HPLC and UV methods for determination hyoscine butyl bromide and compare the analytical results with official BP methods.

Chapter two

2MATERIALS, AND METHODS

2.1 MATERIALS

2.1.1 Chemical solvents and reagents

- Sodium hydroxide pellets,SD FINE-CHEM LIMITED (SDFCL), Industrial Estate, 248, Worll Road, Mumbai-30.
- Hydrochloric acid(UN1789), CARLO ERBA Reagent Group.
- Sodium lauryl sulphate, central drug house P Ltd, 7/28 daryaganj new Delhi110002 (INDIA).
- Silver nitrate, central drug house P Ltd, 7/28 daryaganj new Delhi110002 (INDIA).
- Methylene chloride(Dichloromethane),D/1852/17,Fisher Scientific UK.
- Acetonitrile HPLC Grade , High purity chemicals, Duksan reagent
- Methanol HPLC Grade, High purity chemicals, Duksan reagent.
- Water for HPLC,High purity chemicals, Duksan reagent.
- Anhydrous ethanol,Alphachemika India..
- Chloroform LR ,Alpha chemika India.

2.1.2 STANDARD

Hyoscine butyl bromide working standard from UNIMEDPharmaceutical Khartoum, Sudan Batch NO. : 201611

MFG Date: 01/08/2014 Exp Date: 01/08/2019

Loss on drying: 0.51%Assay: 100.82%

2.1.3 INSTRUMETS

2.1.3.1 High performance liquid chromatography spectroscopy

High performance liquid chromatography spectroscopy (HPLC) was recorded on YL-9330instrument , with UV/Visible ,UV detectors , low pressure gradient pump , Auto sampler and PC control.

2.1.3.2 Ultraviolet –Visible Spectrophotometer (UV-VIS)

Ultraviolet spectral data analyses were carried out uses UV-VIS_1800 series Spectrophotometer (Shamdzu)double beamwithPC control

2.1.3.3 Infrared Spectrophotometer (IR)

Infra-Red spectroscopy was recorded on IR Affinity-1 instrument (Shamdzu, Japan) using KBr disc**.**

2.1.4 General equipments

- Electronic balance, Æ ADAM, Adam Equipment.
- pH meter, professional meter PP-20, Sartorius.
- Melting point apparatus, ⎪SMP30⎪, Stuart.
- Ultra-sonication (Degasser), BANDELIN SONOREX.
- Filtration system, JEBIVAK, J.B.SawantEngg. Put.ltd, INDIA.
-

2.1.5 Glassware

All glass wares are Pyrex type.

2.2 METHODS

2.2.1 Identification test for hyoscine butyl bromide

The FTIR spectra of reference standard, test sample of hyoscine butyl bromide were recorded with FT-IR Affinity-1 instrument (Shamdzu, Japan) by KBr disk method, prepared by finely grinding 1part of hyoscine butyl bromide with about 200 part of dried potassium bromide. The mixture was compressed under 10 tons with pressure between 60-80 kg/inch manually in vacuum pressure Perkin Elmer. FT-IR computerized spectrometer was used to obtain IR spectrum, the wave length range of 500 to 4000 cm1 scanning speed (2mm/sec), resolution $2cm^{-1}$ using detector IR infinity-1

2.2.2 Effect of solvent upon UV spectrum of hyoscine butyl bromide

Accurate weight 0.1001g of hyoscine butyl bromide was dissolved in each dry 100 ml volumetric flask, with solvent (water, methanol, Acetonitrile) and scanned in range 200-400 nm.

2.2.3 Effect of acid and base upon UV spectrum of hyoscine butyl bromide

Accurate weight 0.1001g of hyoscine butyl bromide was dissolved in 100 ml volumetric flask with0.1M hydrochloric acid or 0.1M sodium hydroxide used as above to have a concentration of 10µg/ml both are scanned over the range of 200 to 400nm.

2.2.4 Effect of pH on the UV spectrum of hyoscine butyl bromide

Accurately weight of 0.1000 g of hyoscine butyl bromide transferred into 100 ml volumetric flask with buffer, the volume was complete to the mark with same solvent. solution was scanned in the range over 200 to 400 nm, in pHrange from 1 to14.

2.3Method Validation

2.3.1 Selection of solvent

Hyoscine butyl bromide was freely soluble in water and methylene chloride, sparingly soluble in anhydrous ethanol, so after study the solubility profile of hyoscine butyl bromide in different solvents, water as solvent was selected as common solvent for developing spectral characteristics.

2.3.2 Preparation of standard solution

In UV method 1.000 g of hyoscine butyl bromide standard was weighed accurately by using sensitive balance, dissolved in 100ml volumetric flask and completed to the mark with water. The solution was sonicated for 10 minutes. The stoke solution was kept at room temperature.

In HPLC method the same weight was taken and dissolved in 25 ml volumetric flask with0.001M hydrochloric acid and shaked by mechanical shaker forn10 minutes. The solution was kept at room temperature and completed to 100 ml with water.

2.3.3 Linearity and range of the two methods

From stock solution of series0.1, 0.4, 0.6, 0.8, 1.0, 1.2,1.5, 1.8v/v were prepared in separate 25 ml volumetric flask, sonicated and completed to the mark with water , for determination of linearity of UV method at 257 nm.

In the HPLC method from stock solution a series of0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8v/v aliquots were preparein separate 25 ml volumetric flask, sonicated and completed to the mark with 0.001M HCL and transferred the different concentration solutions to auto sampler unit in HPLC-

YL9330 instrument. 10µl from each concentrationsolutions was injected into the column .

2.3.4 Accuracy and recovery of the two methods

2.3.4.1 Preparation of hyosine butyl bromide standard stock solution

In HPLC method 2.0344 g of hyoscine butyl bromide standard was weighed and transferred into the 100 ml volumetric flask, dissolved with 60 ml HCl 0.001M andshaked by mechanical shaker for 10 minutes.The solution was left to cool down at room temperature, completed to the mark with water ,and from solution transferred10, 12.5 ,15 ml aliquots were transferred into25 ml volumetric flask, to prepare80, 100 and 120% of label concentration.

2.3.5 Repeatability

From stock ofhyoscine butyl bromide standard solution 0.5 mg/ml was prepared in 100 ml volumetric flask and the volume completed to the mark with water,10µl was taken from standard solution and which was then repeatedly auto injected for five times in HPLC column.

2.4Design and Validated of HPLC and UV Methods

2.4.1 The development Method

In this work HPLC method for determination of hyoscine butyl bromide was developed and validated. The validationof the method was done by selection of suitable solvents such as methanol and acetonitrile, suitable mobile phase (2g of Sodium dodecyl acetate in mixture of (370 ml of 0.001M HCL + 680 ml of Methanol)with columns C18, and detection wavelength and analyte concentration. The detection wave length of 210nm was selected after scanning the standard solution along the range of 200-400nm by using UV detector, and The developmentof the HPLC

method was done by selection of modified mobile phase (1g of Sodium dodecyl acetate in mixture of (250 ml of 0.001M HCL + 750 ml of Methanol) with samecolumn , detection wavelength and analyte concentration. The development of the UV method was done by selection suitableof solvent (water).

2.4.2 **Chromatographic system**

The liquid chromatography was equipped with 15cm*3.9mm column that contains (C18), the flow rate was about 2.0 ml per minute and the separately inject volumesfrom 0.1 to 0.8mg/ ml and the detection wavelength was 210 nm,

Chapter Three

3.RESULTS AND DISCUSSION

3.1 Identification

3.1.1 Identification test for hyoscine butyl bromide

The IR spectrum of hyoscine butyl bromide showed characteristic peaks at the wave number:1720 cm^{-1}indicating the presence of $C=O_{st.vib}$carbonyl group,3355cm^{-1}1/2$OH_{st.vib}$due to hydroxyl group,3060cm^{-1} for $C\text{-}H_{st.vib}$ Aromatic,2855-2950 $cm^{-1}$$C\text{-}H_{st.vib}$for saturated,1200 $cm^{-1}$for C-O bending, 1475-1600 cm^{-1} foraromatic ring $C=C_{st.vib}$, 700-800 cm^{-1}d for mono substituted benzene ring.

IR spectrum ofhyoscine butyl bromideis shown in Figure (3.1)

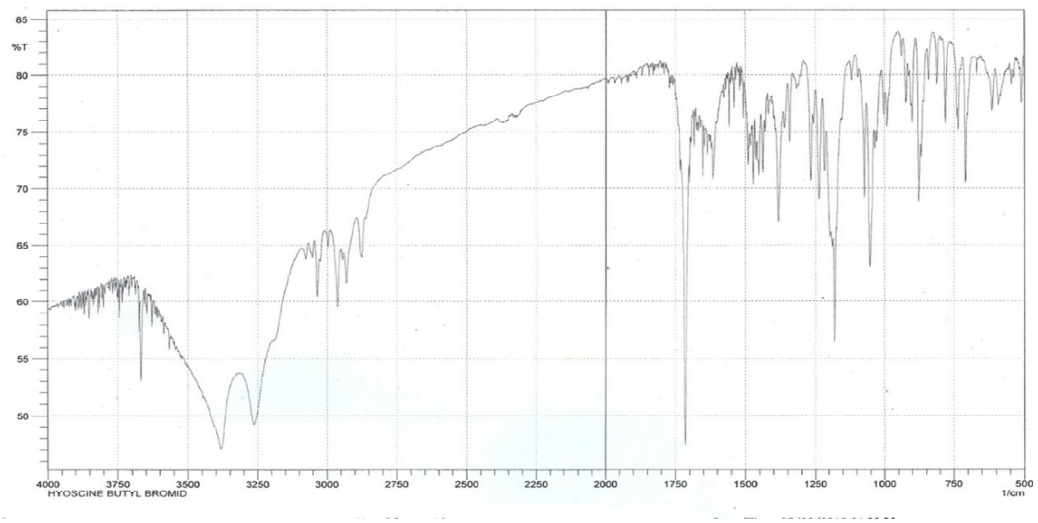


Figure (3. 1): IR spectrum of hyoscine butyl bromide

3.1.2 Effect of solvent upon UV spectrum of hyoscine butyl bromide

Water and many organic solvents such as methanol, ethanol, acetonitrile, etc, are used for this purpose. Theses solvents dissolved the sample well, water selected as suitable solvent for developing characteristics of the drug. The selection assessing the solubility of drug in different solvents is shown in Figures (3.2) (3.3)

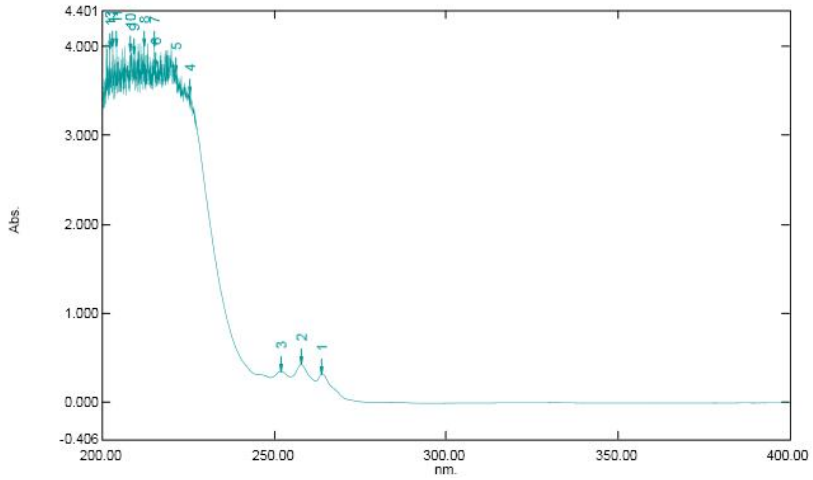


Figure (3.2): Methanol solvents effect on UV spectrum of hyoscine butyl bromide

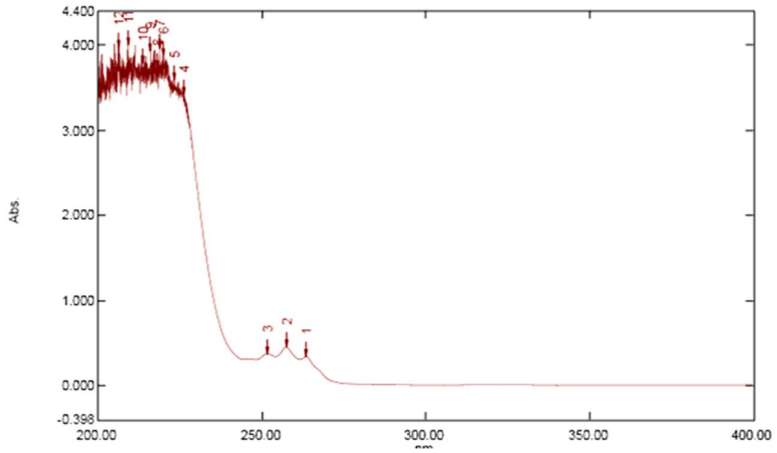


Figure (3.3): water solvents effect on UV spectrum of hyoscine butyl bromide

3.1.3 Effect of acid and base upon UV spectrum of hyoscine butyl bromide

Hyoscine butyl bromide maximum absorbance in acid media at wavelength 257nm is 0.425(Figure (3.4)), The maximum absorbance in base media at wavelength 257nm is also 0.425 nm, as shown in Figure (3.5)

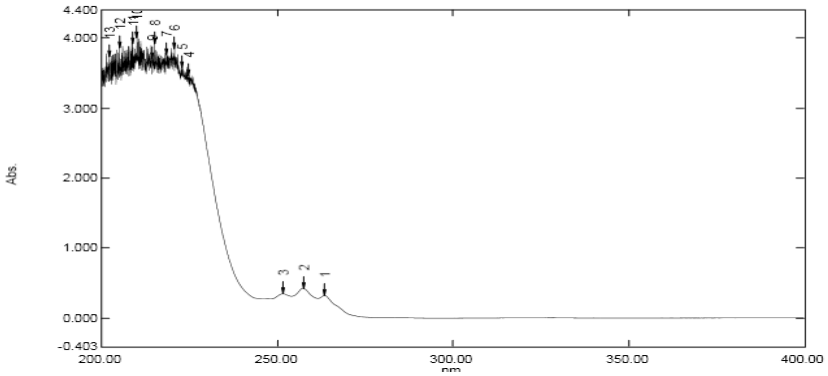


Figure (3.4) Effect acid media upon UV spectrum of hyoscine butyl bromide

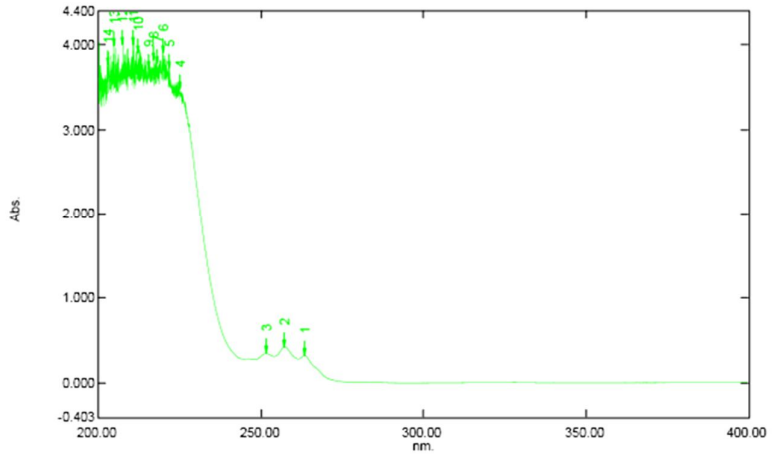


Figure (3.5) Effect basic media upon UV spectrum of hyoscine butyl bromide

3.1.4 Effect of pH upon UV spectrum of hyoscine butyl bromide

The study of the effects of the pH on absorption of the sample at the UV region at high and low pH revealed that there was no peak absorption at pH lower than 3 and higher than pH 12 at wavelength of 257nm. Themaximum absorption peak was clear at the pH 7at wave length 257nm, as shown in table (3.1) and Figures (3.6), (3.7) ,(3.8)and (3.9).

Table (3.1) effect of pH upon UV spectrum of hyoscine butyl bromide at 257 nm

pH	Abs
3	0.434
4	0.432
5	0.452
6	0.456
7	0.468
8	0.431
9	0.445
10	0.500
11	0.541
12	0.608

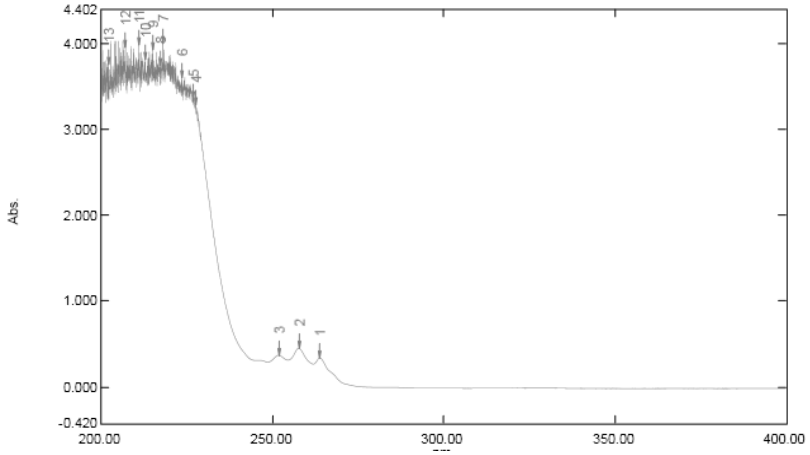


Figure (3.6): pH (5)

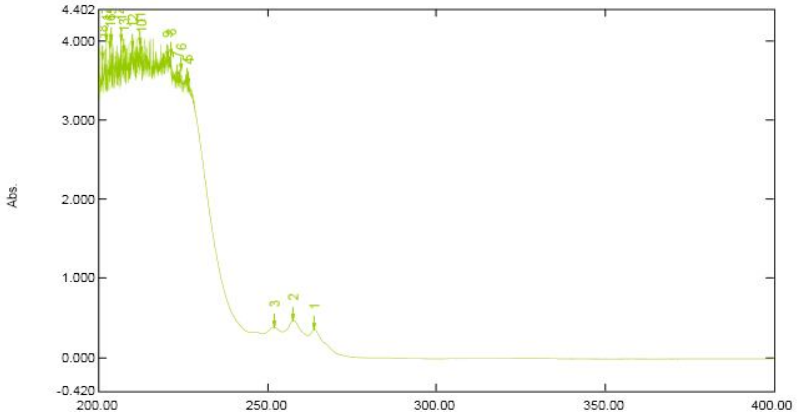


Figure (3.7): pH (7)

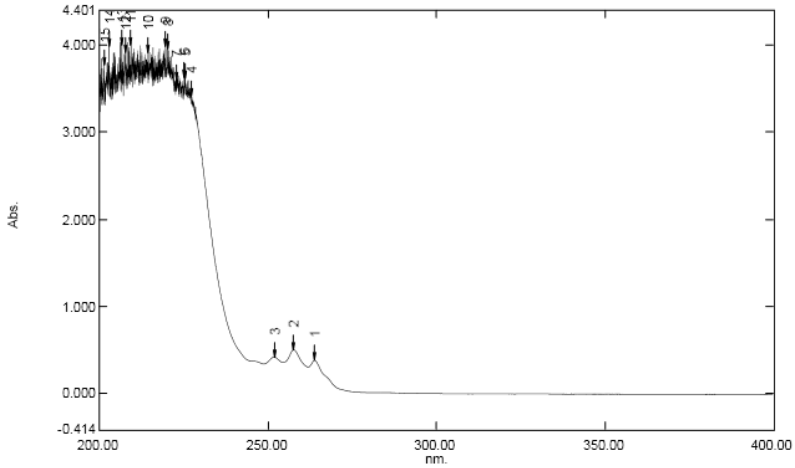


Figure (3.8): pH (10)

3.2 Methods Validation

3.2.1. Development and Validation of High Performance Liquid Chromatography Methods for determination of hyoscine butyl bromide

The traditional approach to HPLC optimization is to perform an experiment by changing control variableseach time; such method can frequently require very large number of experiments to identify the optimal conditions. Recently computer hyphenated to HPLC instrument has addressed the problem using factorial design strategies. In this work using column C18,the optimum flow rate of mobile phase was 2ml/min, The developed HPLC method reduced the retention time from14.26 minutesin Figures (3.9), to 9.0 minutes in figure (3.10)

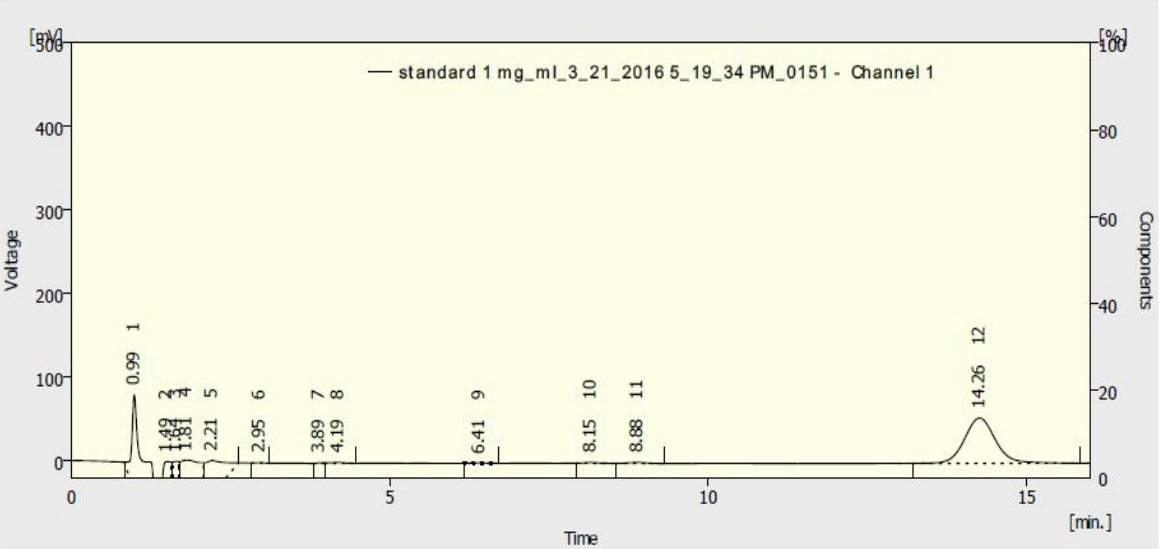


Figure (3.9) Typical HPLC chromatogram for hyoscine butyl bromide standard using developed method

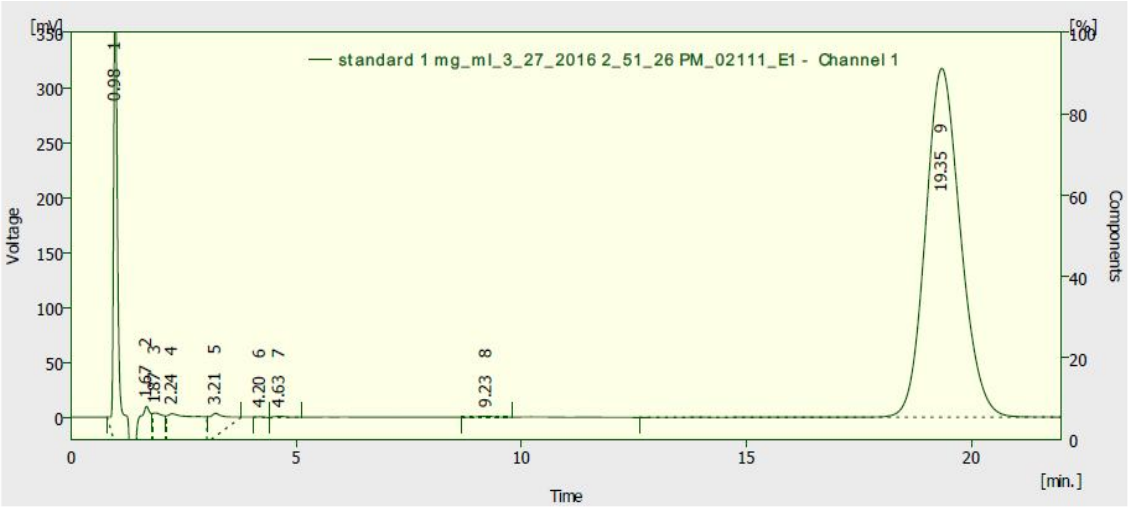


Figure (3.10) Typical HPLC chromatogram for hyoscine butyl bromide standard using official method BP

3.2.1.1 Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity of detector response for hyoscine butyl bromide was established by analyzing serial dilutions of a stock solution of the working standard. Eight concentrations of0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8mg/ml ofhyoscine butyl bromide wereprepared and analyzed. The linearity graph was plotted as shown in Figure (3.11)

Table (3.2) calibration curve of hyoscine butyl bromide

Standard Concentration(mg/ml)	Area
0.1	1744.21
0.2	3513.402
0.3	5522.174
0.4	6881.897
0.5	8699.3074
0.6	10493.254
0.7	12596.628
0.8	13759.284

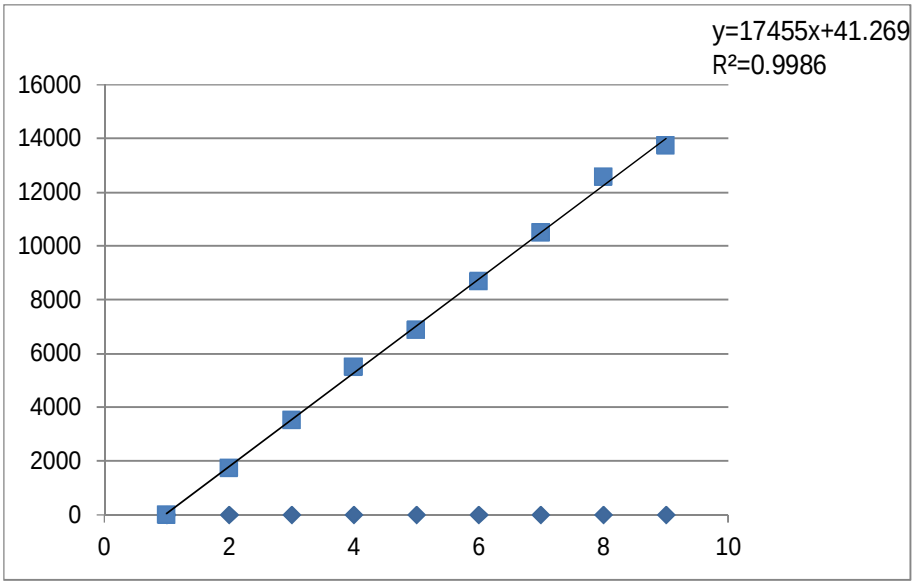


Figure (3.11) Linearity curve of hyoscine butyl bromide

3.2.1.2 Accuracy

The accuracy of the method was assessed by determination of recovery for five concentrations covering the range of the method. The amount of hyoscine butyl bromide was recovered in the presence of placebo interference, was calculated. The mean recovery of hyoscine butyl bromide was 99.89% and the RSD% was less than 2% which is satisfactory as shown in Table (3.3)

Table (3.3) Accuracy ofhyoscine butyl bromide (HPLC yl-9330)

Sample	**Area**	**Theoretical**	**conc. Calculated**	**Assay**
0.4	7774.155	80%	0.445382	101.3455
0.5	9138.027	100%	0.523519	103.7038
0.6	10487.625	120%	0.600837	100.1395
			Mean	101.7296
			SD	1.4802
			RSD%	1.45503

3.2.3.3 System suitability

The obtained results of system suitability showed that the HPLC system in Table (3.4)

Table (3.4) System suitability data of hyoscine butyl bromide

NO	area of standard	Retention time	Tailing factor
1	1740.13	14.45	1.022
2	1744.21	14.34	1.006
3	1745.95	14.20	0.976
4	1747.46	14.16	0.942
5	1747.98	14.09	0.929
Avg	1745.1		
SD	2.8324		
RSD%	1.623		

3.2.3.4 Detection limit and quantitation limit

The limit of detection and limit of quantitation of hyoscine butyl bromide was determined by using the signal to noise ratio approach as defined in ICH guidelines. According to the determined signal to noise ratio the limit of detection and limit of quantitation for hyoscine butyl bromidewas 0.0393µgµg/ml and0.119µg/m, respectively.

3.3 ValidationHPLC method resultof accuracy of three concentrations of hyoscine butyl bromide

the chromatograms record for 80% , 100% and 120% for hyoscine butyl bromide were recorded in figure 3.13,3.14 and 3.15

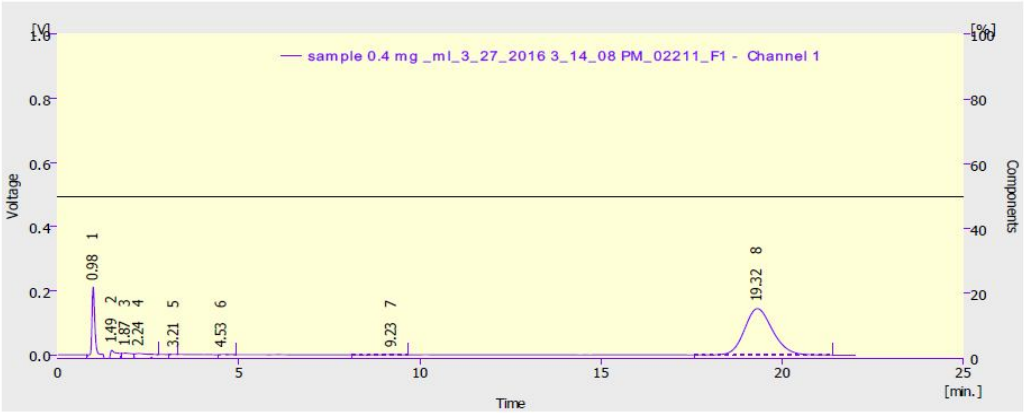


Figure (3.12) HPLC chromatogram for (80%) of hyoscine butyl bromide

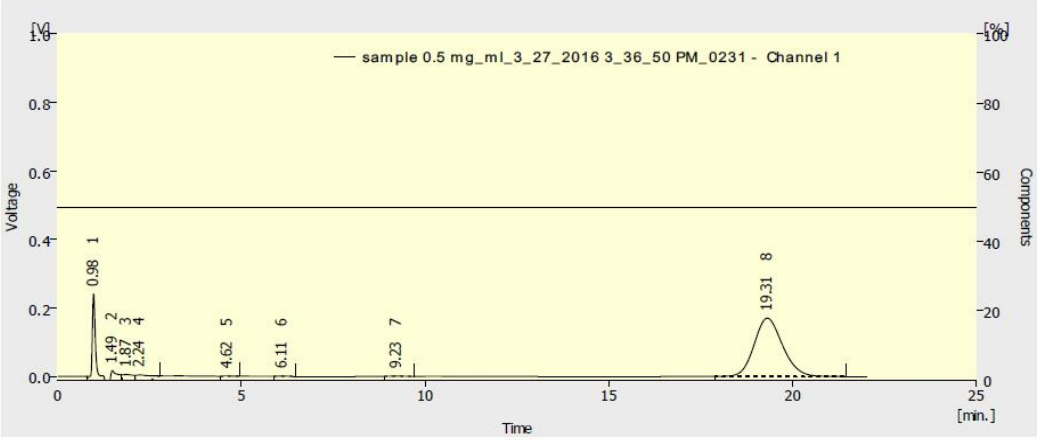


Figure (3.13) HPLC chromatogram for (100%) of hyoscine butyl bromide

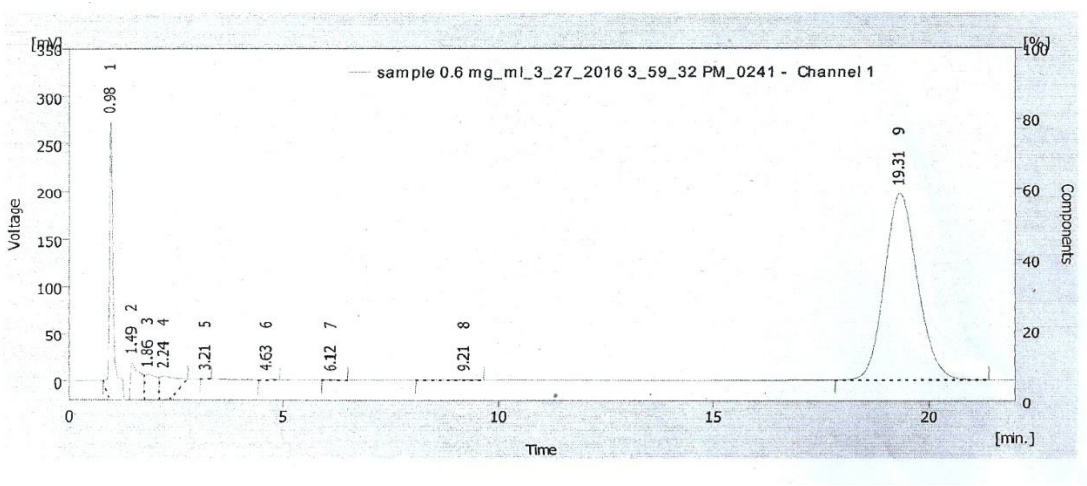


Figure (3.14) HPLC chromatogram for (120%) of hyoscine butyl bromide

3.3.1 Assay of samples

Comparison of HPLC assay of hyoscine butyl bromide between the official and the developed validated methodis shown in Table (3.5).

Table (3.5): The assay of hyoscine butyl bromide

NO	Sample conc.	HPLC official method	HPLC developed method
1	0.4 (80%)	101.3455	102.176
2	0.5 (100%)	103.7038	100.332
3	0.6 (120%)	100.1395	100.353
Avg		101.7296	100.95367
SD		1.4802	1.2335
RSD%		1.45503	1.2219

3.4 Development and Validation of UV spectrophotometric of hyoscine butyl bromide

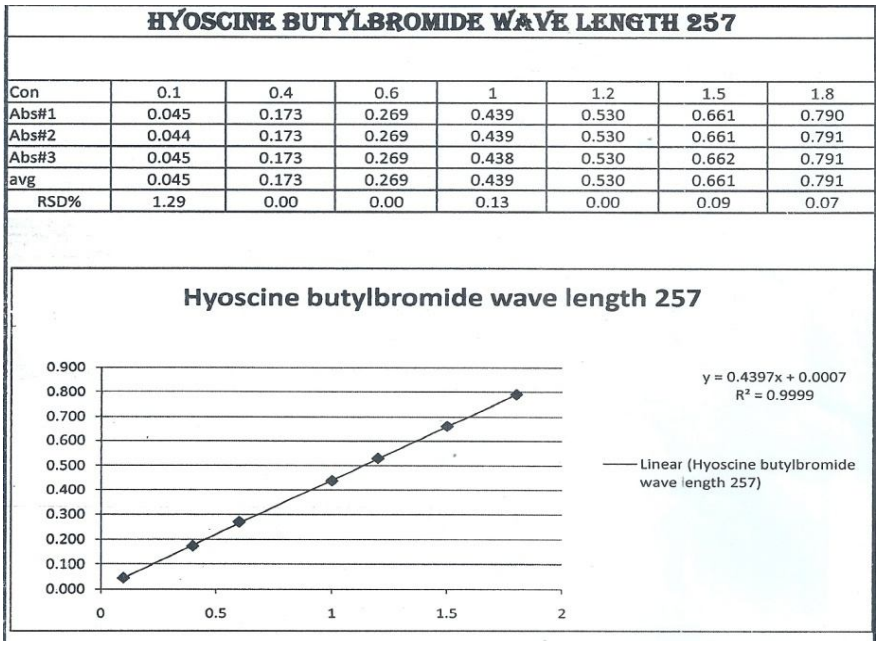
3.4.1 Determination of linearity and range

The linearity for hyoscine butyl bromide was determined in term of correlation coefficient, which equals0.9999,indicating the linearity of the method. UV Spectrophotometer absorption of hyoscine butyl bromide standard solution gave linearity in the range between 0.1mg/ml and 1.8 mg/ml

Table (3.6) standard curve of hyoscine butyl bromide

Conc. mg/ml	UV absorbance 250 nm
0.1	0.045
0.4	0.173
0.6	0.269
1.0	0.439
1.2	0.530
1.5	0.661
1.8	0.791

Figure (3.15) calibration curve of hyoscine butyl bromide standard solution



HYOSCINE BUTYLBROMIDE WAVE LENGTH 257

Con	0.1	0.4	0.6	1	1.2	1.5	1.8
Abs#1	0.045	0.173	0.269	0.439	0.530	0.661	0.790
Abs#2	0.044	0.173	0.269	0.439	0.530	0.661	0.791
Abs#3	0.045	0.173	0.269	0.438	0.530	0.662	0.791
avg	0.045	0.173	0.269	0.439	0.530	0.661	0.791
RSD%	1.29	0.00	0.00	0.13	0.00	0.09	0.07

3.4.2 Precision

The precision (system method) of proposed method was evaluated the assay were performed by repeatability (intraday) and intermediate reported as RSD%. Tabulated in Table (3.6) shown the repeatability precision of system method

Table (3.7) Repeatability precision of system method of (1.0 mg/ml)

Run	Abs at 257 nm
1	0.439
2	0.438
3	0.439
Avg	0.438
RSD%	0.1863

3.5 Conclusion and Recommendation

Validation of HPLC method was applied as indicating method recommended for routine work. The assay result of the developed methods was found to be similar to those obtained by official methods. Thus the proposed method for the estimation of hyoscine butyl bromidedosage form was found to be rapid, simple, accurate, precise and economical. High percentage of recovery shows that the method was free from the interference. The verification of the validity of your method hyoscine butyl bromide assay .But the method of using the HPLC assay device was more accurate, and the accuracy of the linear method indexation by the UV, in the case of non availability of the device can be used as alternative UVand get similar results. The developed UV Spectrophotometer method is suitable for routine analytical procedure for the analysis of hyoscine butyl bromide tablets, it is inexpensive and is time consuming and gave comparable results from HPLC can therefore be used in the event of unavailability of HPLC Developed HPLC in laboratory. Method recommended for applying as routine analysis in quality control laboratories for the analysis of the hyoscine butyl bromide, and the shorter time than the official method and simple, safe, rapid, accurate and not expensive. Follow up the development of new methods for the estimating the hyoscine butyl bromide in all pharmaceutical forms because it is one of the important pharmaceutical treatment of gout.

3.6 Reference

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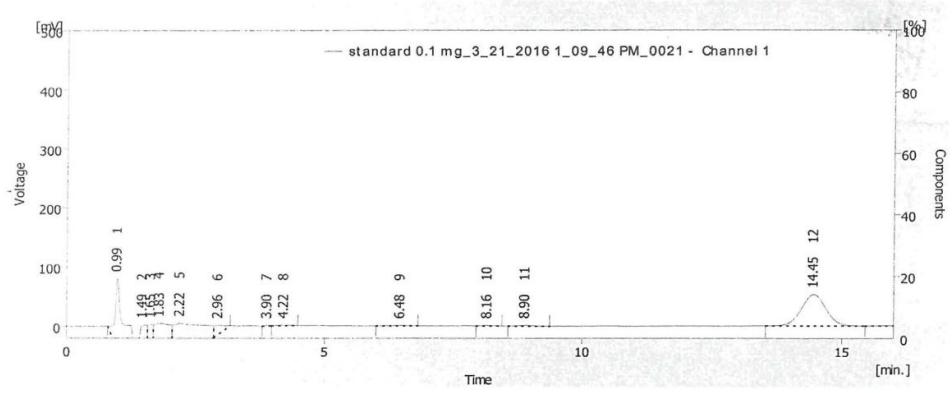
Appendix

Sample Info:

Sample ID	: standard 0.1 mg	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

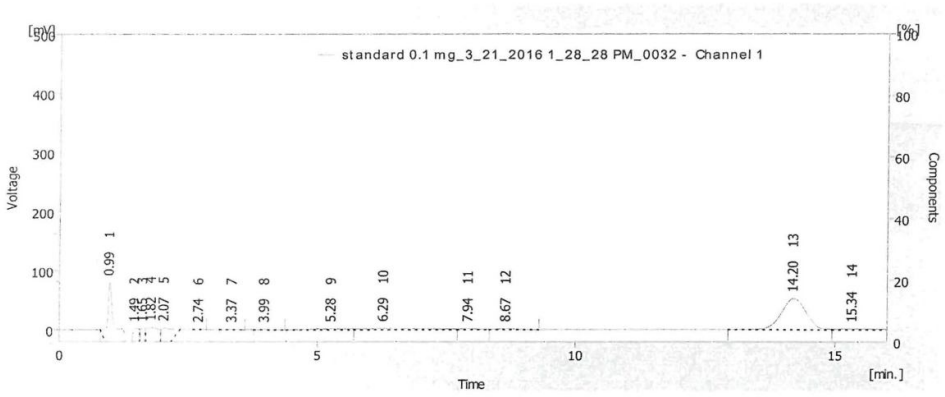


Result Table (Uncal - standard 0.1 mg_3_21_2016 1_09_46 PM_0021 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.990	0.800	1961.735	119.413	19.8		509	1.551	0.000
2	1.490	1.350	1111.136	106.441	11.2		394	0.859	2.107
3	1.650	1.583	661.803	97.141	6.7		1174	0.850	0.651
4	1.827	1.697	1840.359	88.221	18.5		137	1.410	0.434
5	2.220	2.063	2244.666	64.098	22.6		42	2.574	0.396
6	2.957	2.870	192.888	15.021	1.9		483	1.769	0.774
7	3.900	3.797	6.873	1.085	0.1		6191	1.045	2.569
8	4.220	3.980	21.674	1.217	0.2		1173	0.972	0.929
9	6.480	6.003	14.157	0.737	0.1		2831	0.826	4.625
10	8.163	7.943	12.216	0.908	0.1		7864	1.096	3.946
11	8.900	8.567	26.060	1.352	0.3		4876	1.200	1.682
12	14.450	13.537	1740.126	52.808	17.5		4447	1.022	8.085
13	16.570	15.450	90.313	1.438	0.9		1685	1.006	1.713
	Total		9924.007	549.879	100.0				27.911

Sample Info:

Sample ID	: standard 0.1 mg	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

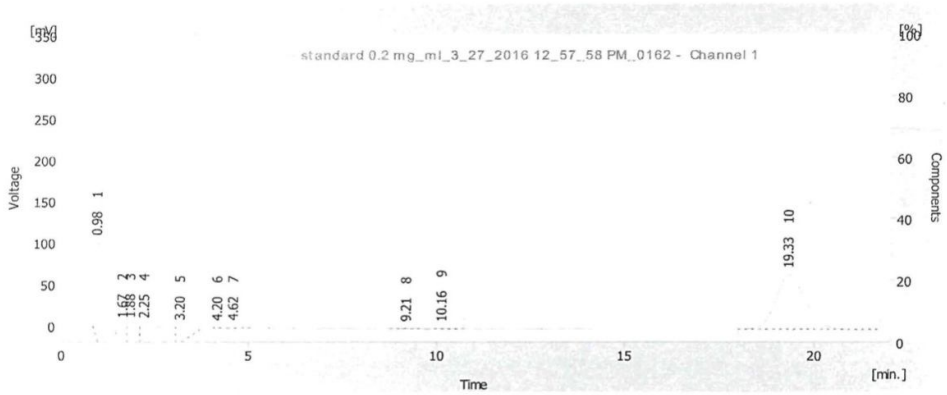


Result Table (Uncal - standard 0.1 mg_3_21_2016 1_28_28 PM_0032 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.987	0.800	1960.490	118.632	26.2		505	1.573	0.000
2	1.490	1.347	1040.914	99.314	13.9		394	0.859	2.121
3	1.647	1.583	546.587	82.291	7.3		1241	0.868	0.645
4	1.817	1.693	1091.624	65.022	14.6		203	1.216	0.489
5	2.067	1.993	495.998	36.434	6.6		164	2.500	0.434
6	2.737	2.613	3.287	0.478	0.0		3230	1.031	1.603
7	3.373	3.120	11.237	0.720	0.1		716	0.950	1.832
8	3.993	3.773	13.741	0.955	0.2		1882	1.297	1.425
9	5.280	4.400	100.100	1.900	1.3		172	0.763	1.305
10	6.293	5.710	211.028	2.657	2.8		61	1.717	0.422
11	7.937	7.713	43.250	1.784	0.6		1947	1.396	0.838
12	8.670	8.337	42.776	1.737	0.6		3042	1.165	1.091
13	14.197	12.943	1744.210	52.782	23.3		4183	0.976	7.355
14	15.340	14.937	189.368	1.718	2.5		507	2.843	0.636
	Total		7494.610	466.422	100.0				20.196

Sample Info:

Sample ID	: standard 0.2 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

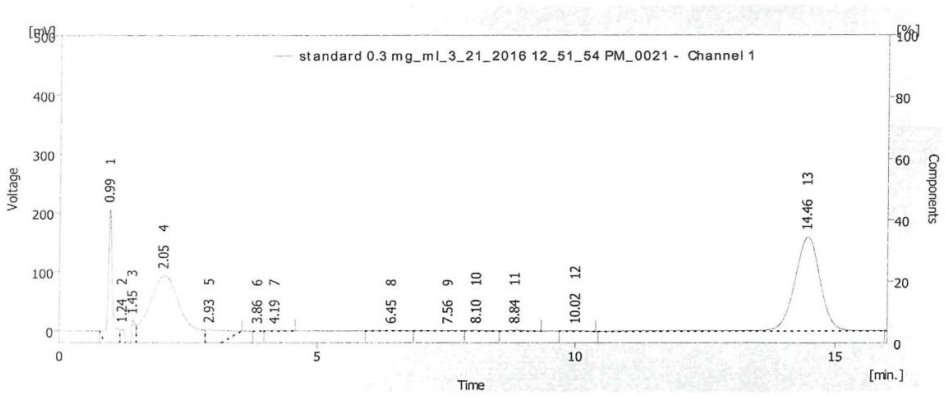


Result Table (Uncal - standard 0.2 mg_ml_3_27_2016 12_57_58 PM_0162 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.977	0.850	1466.189	121.174	14.1		607	2.407	0.000
2	1.673	1.337	1433.731	68.502	13.8		137	0.634	1.912
3	1.880	1.760	1252.198	62.228	12.1		160	1.458	0.355
4	2.250	2.110	2167.369	50.478	20.9		31	3.381	0.337
5	3.203	3.057	496.391	18.592	4.8		109	2.364	0.674
6	4.200	4.053	7.122	0.532	0.1		1650	1.293	1.217
7	4.623	4.450	23.424	1.224	0.2		1066	1.948	0.866
8	9.213	8.767	10.315	0.412	0.1		3374	1.147	7.664
9	10.160	9.763	7.453	0.290	0.1		3347	1.155	1.420
10	19.333	17.990	3513.402	65.091	33.9		3030	1.177	8.729
	Total		10377.595	388.523	100.0				23.174

Sample Info:

Sample ID	: standard 0.3 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

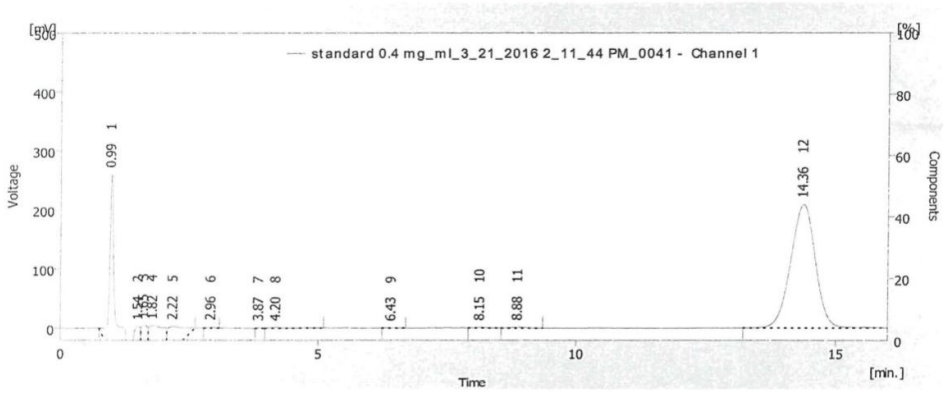


Result Table (Uncal - standard 0.3 mg_ml_3_21_2016 12_51_54 PM_0021 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.990	0.787	1918.741	246.911	10.2		782	1.186	0.000
2	1.243	1.187	675.088	94.057	3.6		629	1.294	1.495
3	1.447	1.347	835.550	123.324	4.4		852	0.852	1.028
4	2.050	1.510	8896.216	169.554	47.4		20	1.231	0.602
5	2.930	2.840	782.986	33.339	4.2		95	3.759	0.586
6	3.863	3.763	7.134	0.994	0.0		3844	1.250	1.291
7	4.193	3.973	17.663	1.088	0.1		1304	1.068	0.927
8	6.453	5.930	15.313	0.751	0.1		2743	0.953	4.734
9	7.557	6.873	15.935	0.463	0.1		841	0.724	1.441
10	8.103	7.863	23.339	1.187	0.1		5116	1.396	0.733
11	8.843	8.533	29.090	1.441	0.2		4413	1.032	1.506
12	10.017	9.687	18.755	0.833	0.1		3254	1.038	1.905
13	14.463	10.447	5522.174	159.234	29.4		4341	0.988	5.642
14	16.517	15.953	18.989	0.481	0.1		4062	0.899	2.151
	Total		18776.973	833.658	100.0				24.040

Sample Info:

Sample ID	: standard 0.4 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

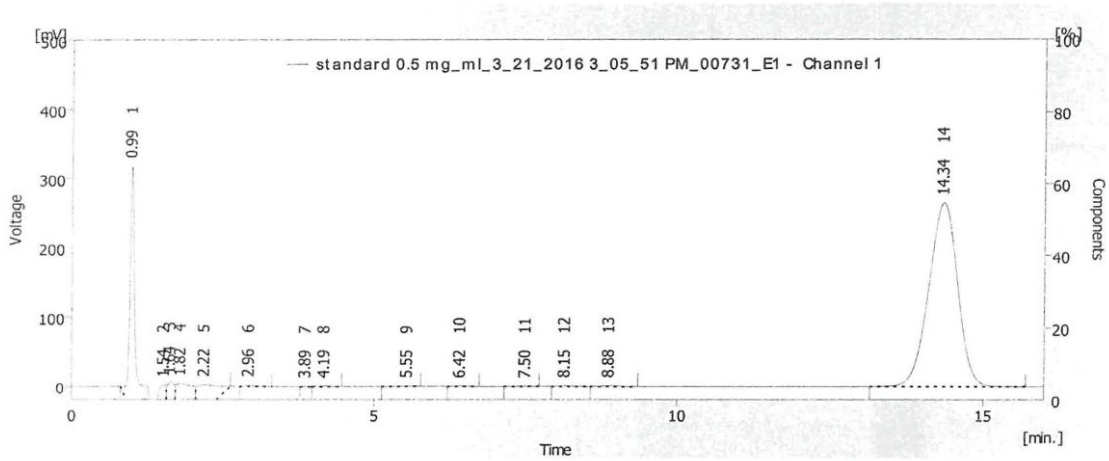


Result Table (Uncal - standard 0.4 mg_ml_3_21_2016 2_11_44 PM_0041 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.993	0.757	2962.673	305.660	21.0		854	1.542	0.000
2	1.537	1.350	1038.909	100.038	7.4		453	0.625	2.565
3	1.647	1.580	782.014	93.161	5.6		731	1.075	0.414
4	1.823	1.723	1477.163	76.108	10.5		137	1.833	0.409
5	2.220	2.090	839.764	39.833	6.0		89	2.051	0.509
6	2.960	2.807	12.086	1.768	0.1		4012	1.030	1.316
7	3.873	3.793	6.985	0.919	0.0		4046	1.300	4.254
8	4.200	3.980	24.622	1.170	0.2		897	2.432	0.814
9	6.427	6.240	3.598	0.258	0.0		4203	1.216	4.664
10	8.147	7.913	15.121	0.954	0.1		6950	1.542	4.380
11	8.883	8.557	28.432	1.423	0.2		4549	1.066	1.610
12	14.363	13.217	6881.897	208.426	48.9		4394	0.988	7.886
13	17.487	17.213	14.544	0.671	0.1		11138	1.115	4.095
	Total		14087.809	830.389	100.0				32.916

Sample Info:

Sample ID	: standard 0.5 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

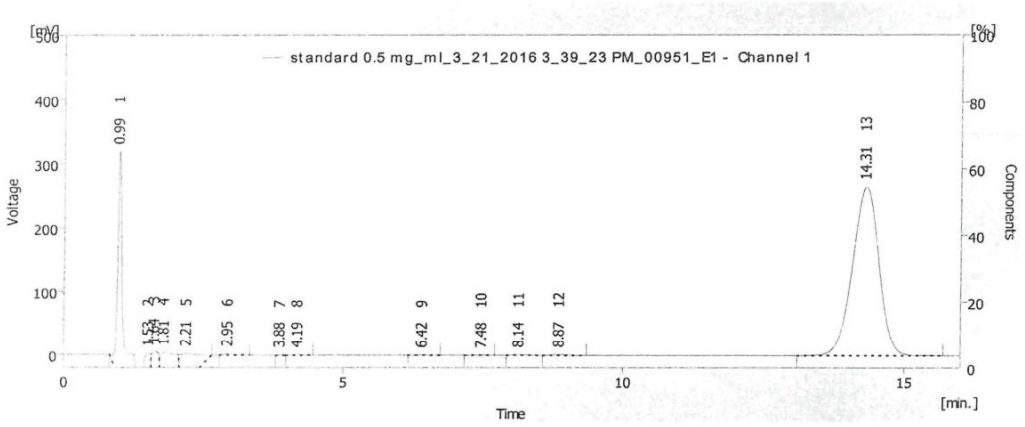


Result Table (Uncal - standard 0.5 mg_ml_3_21_2016 3_05_51 PM_00731_E1 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.990	0.807	3068.465	357.478	19.1		848	2.113	0.000
2	1.540	1.350	1018.638	100.153	6.4		492	0.593	2.667
3	1.643	1.573	832.035	95.296	5.2		665	1.071	0.389
4	1.817	1.723	1418.501	77.406	8.9		155	1.839	0.415
5	2.217	2.067	925.056	40.642	5.8		81	1.867	0.511
6	2.957	2.800	17.165	2.248	0.1		3771	1.091	1.259
7	3.887	3.793	6.745	0.975	0.0		4481	1.250	4.390
8	4.193	3.987	17.760	1.140	0.1		1082	1.000	0.829
9	5.547	5.120	5.934	0.304	0.0		1365	0.781	2.444
10	6.420	6.207	7.095	0.483	0.0		3964	1.164	1.737
11	7.497	7.143	4.898	0.300	0.0		4982	0.796	2.593
12	8.147	7.923	14.505	0.922	0.1		6950	1.586	1.598
13	8.880	8.567	28.054	1.418	0.2		4546	1.090	1.602
14	14.340	13.143	8660.023	263.973	54.0		4380	0.968	7.857
	Total		16024.874	942.738	100.0				28.291

Sample Info:

Sample ID	: standard 0.5 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

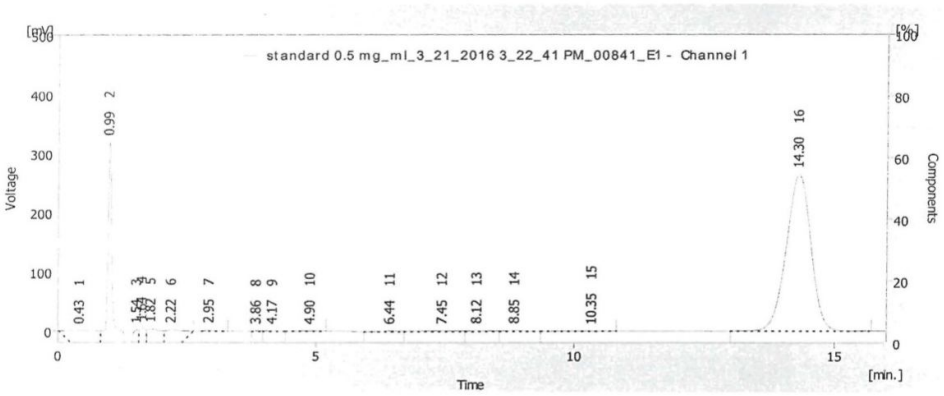


Result Table (Uncal - standard 0.5 mg_ml_3_21_2016 3_39_23 PM_00951_E1 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.990	0.823	3020.370	356.524	18.8		924	2.333	0.000
2	1.533	1.347	1027.458	101.812	6.4		488	0.604	2.671
3	1.640	1.570	844.232	96.671	5.2		662	1.071	0.402
4	1.813	1.720	1468.621	78.781	9.1		149	1.875	0.409
5	2.213	2.070	961.007	42.182	6.0		77	1.988	0.500
6	2.953	2.797	16.839	2.244	0.1		3993	1.061	1.242
7	3.880	3.793	6.376	0.965	0.0		5198	1.237	4.620
8	4.193	3.973	18.085	1.157	0.1		1243	0.947	0.909
9	6.420	6.160	8.695	0.559	0.1		3466	1.043	4.896
10	7.477	7.163	4.727	0.302	0.0		5090	0.839	2.477
11	8.143	7.913	14.904	0.933	0.1		6748	1.576	1.639
12	8.870	8.567	27.755	1.410	0.2		4635	1.093	1.588
13	14.307	13.097	8676.535	264.759	53.9		4360	0.970	7.855
	Total		16095.603	948.300	100.0				29.209

Sample Info:

Sample ID	: standard 0.5 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

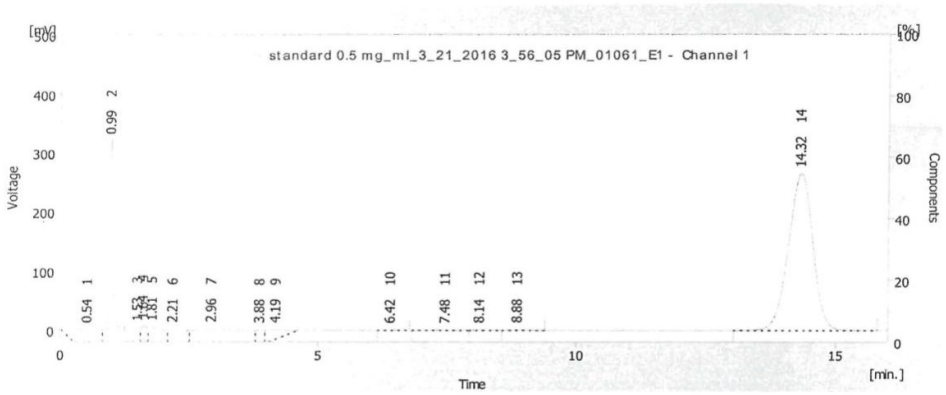


Result Table (Uncal - standard 0.5 mg_ml_3_21_2016 3_22_41 PM_00841_E1 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.427	0.017	1730.345	36.153	9.1		2	1.013	0.000
2	0.990	0.827	4101.549	403.979	21.6		670	1.520	0.739
3	1.537	1.347	1029.283	100.599	5.4		471	0.604	2.513
4	1.643	1.573	832.505	95.352	4.4		665	1.071	0.397
5	1.817	1.723	1425.339	77.305	7.5		152	1.857	0.412
6	2.217	2.070	899.739	40.291	4.7		84	1.875	0.515
7	2.953	2.797	17.014	2.269	0.1		3993	1.059	1.278
8	3.860	3.763	7.517	0.988	0.0		3669	1.350	4.115
9	4.170	3.973	18.603	1.193	0.1		1047	1.110	0.807
10	4.900	4.410	12.769	0.507	0.1		921	0.772	1.261
11	6.443	5.933	38.078	1.442	0.2		1136	0.799	2.194
12	7.453	6.707	86.872	1.492	0.5		224	0.786	0.734
13	8.120	7.880	51.606	1.907	0.3		971	1.382	0.440
14	8.853	8.543	50.402	1.938	0.3		2714	1.301	0.854
15	10.350	9.350	17.851	0.279	0.1		1296	0.718	1.640
16	14.303	13.000	8669.968	263.680	45.7		4358	0.971	3.931
	Total		18989.438	1029.377	100.0				21.831

Sample Info:

Sample ID	: standard 0.5 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

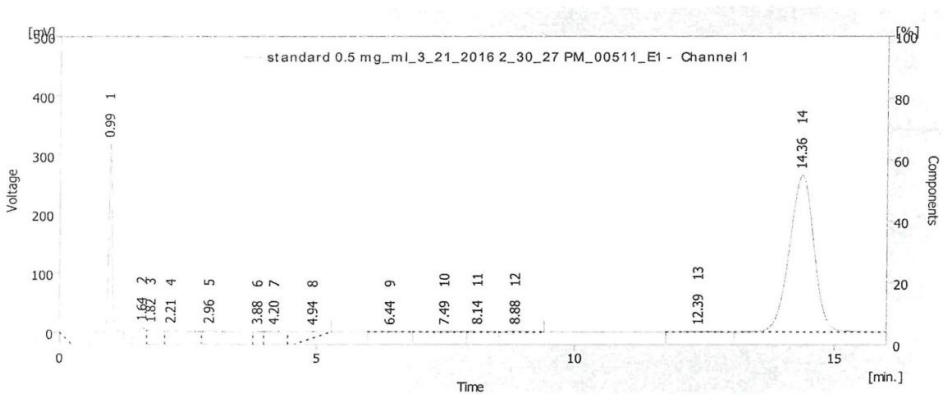


Result Table (Uncal - standard 0.5 mg_ml_3_21_2016 3_56_05 PM_01061_E1 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.540	0.020	1690.726	45.078	6.6		2	0.789	0.000
2	0.990	0.827	4102.923	403.305	16.1		723	1.520	0.594
3	1.530	1.347	1122.193	110.820	4.4		449	0.625	2.483
4	1.640	1.573	972.513	110.748	3.8		662	1.125	0.406
5	1.813	1.723	2177.900	101.831	8.6		128	2.093	0.388
6	2.213	2.100	2043.597	85.248	8.0		151	1.868	0.590
7	2.960	2.523	3853.750	59.551	15.1		30	1.450	0.521
8	3.883	3.790	282.720	26.048	1.1		2486	0.982	0.751
9	4.193	3.973	444.282	15.680	1.7		235	1.417	0.443
10	6.417	6.153	7.819	0.502	0.0		3650	1.080	2.937
11	7.477	6.820	19.330	0.578	0.1		806	0.883	1.438
12	8.143	7.923	24.338	1.190	0.1		4471	1.462	0.868
13	8.877	8.567	32.495	1.521	0.1		4091	1.134	1.411
14	14.320	13.037	8682.357	264.772	34.1		4368	0.970	7.677
	Total		25456.941	1226.872	100.0				20.506

Sample Info:

Sample ID	: standard 0.5 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

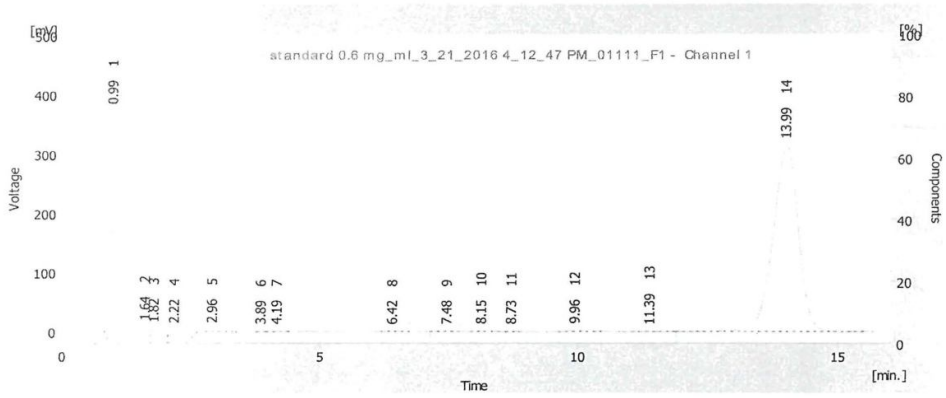


Result Table (Uncal - standard 0.5 mg_ml_3_21_2016 2_30_27 PM_00511_E1 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.987	0.007	5824.120	401.880	20.8		666	0.722	0.000
2	1.643	1.347	2090.772	112.634	7.5		149	0.634	1.905
3	1.817	1.720	2164.224	105.159	7.7		144	1.845	0.304
4	2.213	2.077	3616.613	92.372	12.9		53	2.622	0.436
5	2.957	2.793	3463.265	70.222	12.3		50	3.020	0.515
6	3.883	3.780	507.580	41.369	1.8		1956	1.000	0.916
7	4.203	3.987	868.241	32.321	3.1		463	1.062	0.566
8	4.943	4.447	616.541	10.157	2.2		192	0.829	0.672
9	6.437	5.990	11.092	0.602	0.0		3149	0.916	1.588
10	7.493	6.863	16.618	0.513	0.1		836	0.885	1.417
11	8.143	7.917	24.689	1.222	0.1		4576	1.382	0.859
12	8.877	8.543	33.385	1.541	0.1		4091	1.100	1.419
13	12.393	11.760	16.657	0.333	0.1		1082	1.076	3.420
14	14.360	13.073	8807.654	264.478	31.4		4392	0.971	1.662
	Total		28061.450	1134.803	100.0				15.678

Sample Info:

Sample ID	: standard 0.6 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

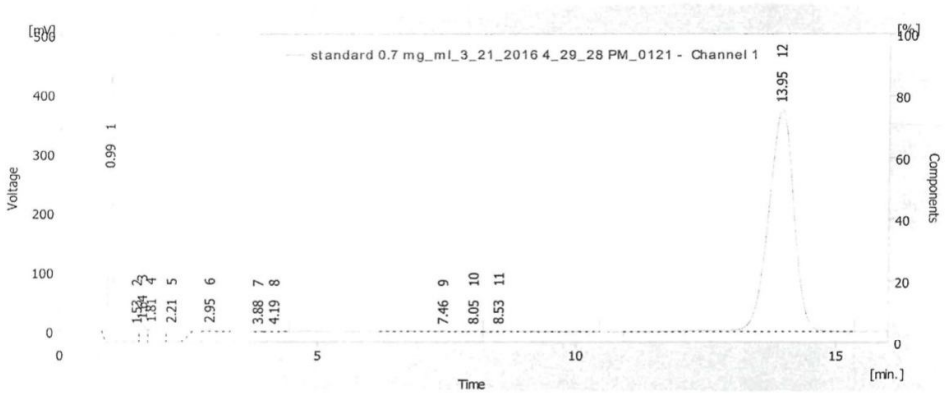


Result Table (Uncal - standard 0.6 mg_ml_3_21_2016 4_12_47 PM_01111_F1 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.993	0.827	3270.460	408.606	18.0		930	2.480	0.000
2	1.643	1.350	1885.972	97.518	10.4		152	0.647	1.967
3	1.817	1.727	1436.090	78.635	7.9		155	1.907	0.311
4	2.217	2.070	962.164	41.918	5.3		77	1.943	0.504
5	2.957	2.800	21.391	2.877	0.1		4002	1.044	1.242
6	3.890	3.793	6.091	0.965	0.0		5822	1.125	4.788
7	4.193	3.973	18.226	1.162	0.1		1213	0.955	0.887
8	6.420	6.180	7.025	0.464	0.0		3753	1.069	4.957
9	7.477	7.143	6.096	0.375	0.0		4701	0.848	2.477
10	8.147	7.920	19.279	1.004	0.1		5883	1.653	1.560
11	8.730	8.600	9.695	1.073	0.1		19628	1.038	1.735
12	9.963	9.637	40.642	1.948	0.2		4489	1.221	2.930
13	11.393	11.037	8.030	0.284	0.0		2915	1.228	1.993
14	13.990	11.887	10493.254	313.154	57.7		4062	0.957	3.024
	Total		18184.416	949.983	100.0				28.377

Sample Info:

Sample ID	: standard 0.7 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

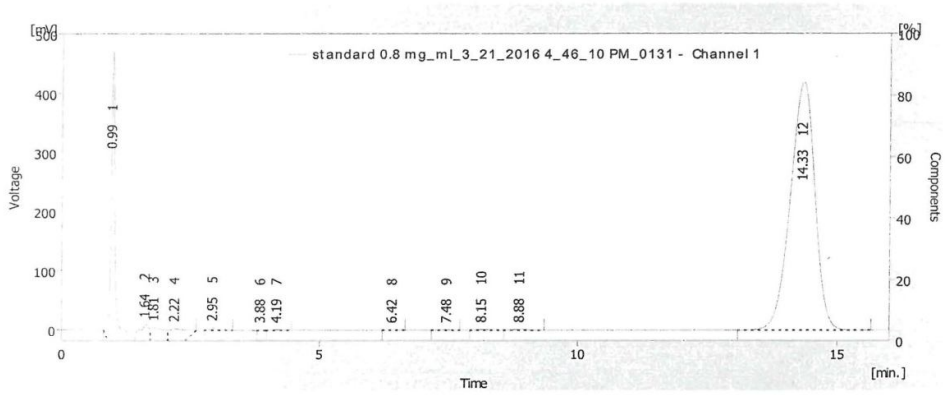


Result Table (Uncal - standard 0.7 mg_ml_3_21_2016 4_29_28 PM_0121 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.990	0.823	3583.594	472.535	17.5		848	2.630	0.000
2	1.533	1.347	965.899	100.099	4.7		554	0.575	2.748
3	1.640	1.560	953.277	98.130	4.7		516	1.063	0.389
4	1.813	1.730	1426.022	76.787	7.0		141	2.160	0.386
5	2.213	2.090	751.519	38.369	3.7		104	1.986	0.543
6	2.953	2.783	23.868	3.225	0.1		3762	1.044	1.401
7	3.880	3.793	6.465	0.962	0.0		4935	1.237	4.494
8	4.187	3.973	18.246	1.173	0.1		1155	0.977	0.862
9	7.457	6.210	41.272	0.825	0.2		1043	0.688	4.630
10	8.047	7.910	9.410	0.978	0.0		13446	0.988	0.985
11	8.533	8.210	47.033	1.390	0.2		5146	3.784	1.295
12	13.950	10.920	12596.628	373.893	61.7		4039	0.944	8.023
	Total		20423.234	1168.366	100.0				25.755

Sample Info:

Sample ID	: standard 0.8 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

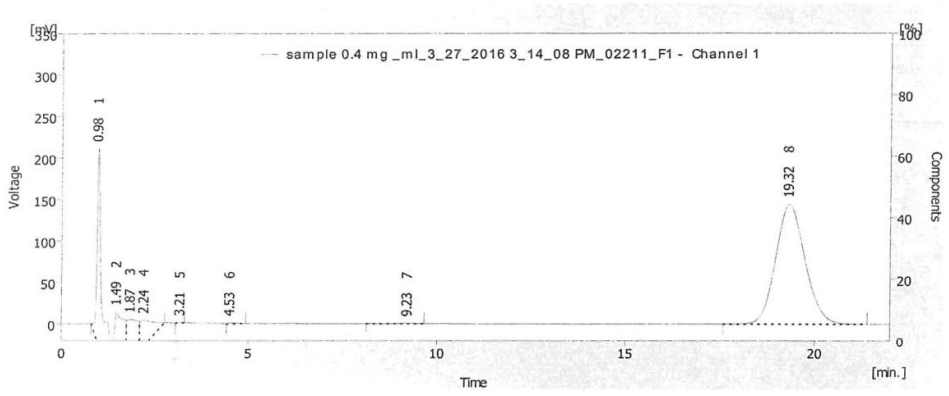


Result Table (Uncal - standard 0.8 mg_ml_3_21_2016 4_46_10 PM_0131 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.993	0.820	3783.809	509.842	17.3		930	2.521	0.000
2	1.643	1.347	1920.633	99.116	8.8		149	0.653	1.950
3	1.813	1.730	1392.554	78.017	6.4		158	2.040	0.305
4	2.217	2.070	866.190	39.544	4.0		89	1.818	0.533
5	2.953	2.783	28.370	3.821	0.1		3762	1.091	1.304
6	3.877	3.797	6.586	0.928	0.0		4458	1.289	4.358
7	4.193	3.977	18.336	1.177	0.1		1185	0.969	0.883
8	6.423	6.217	3.317	0.245	0.0		4449	1.070	5.126
9	7.480	7.170	7.478	0.484	0.0		4959	0.867	2.616
10	8.153	7.917	17.684	0.989	0.1		5590	1.559	1.568
11	8.883	8.570	28.515	1.418	0.1		4453	1.069	1.511
12	14.330	13.083	13759.284	419.052	63.0		4374	0.937	7.806
	Total		21832.755	1154.633	100.0				27.960

Sample Info:

Sample ID	: sample 0.4 mg /ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

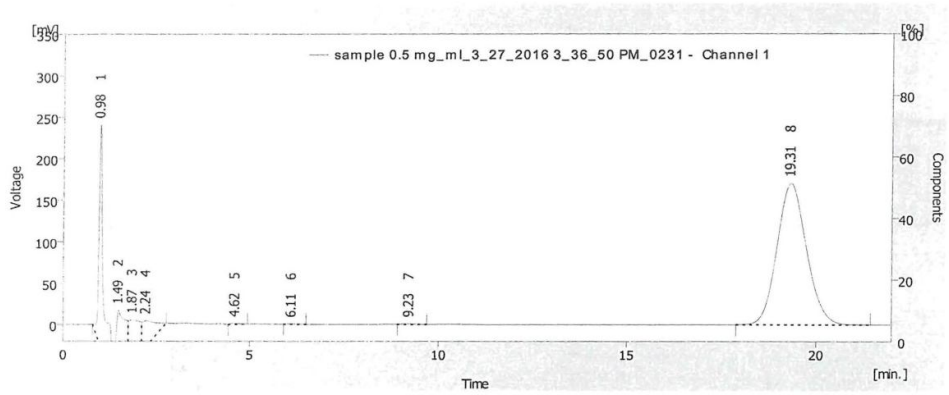


Result Table (Uncal - sample 0.4 mg _ml_3_27_2016 3_14_08 PM_02211_F1 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.977	0.800	2109.312	235.013	16.2		704	2.304	0.000
2	1.493	1.337	1375.875	77.835	10.6		109	1.384	1.440
3	1.870	1.747	1002.544	49.870	7.7		147	1.473	0.635
4	2.240	2.110	730.618	30.243	5.6		61	2.474	0.421
5	3.213	3.073	1.657	0.214	0.0		2059	0.931	1.367
6	4.530	4.430	20.208	1.278	0.2		946	2.700	3.027
7	9.227	8.127	17.353	0.557	0.1		2899	0.699	7.389
8	19.317	17.593	7774.155	144.454	59.7		3025	1.149	9.680
	Total		13031.722	539.464	100.0				23.959

Sample Info:

Sample ID	: sample 0.5 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

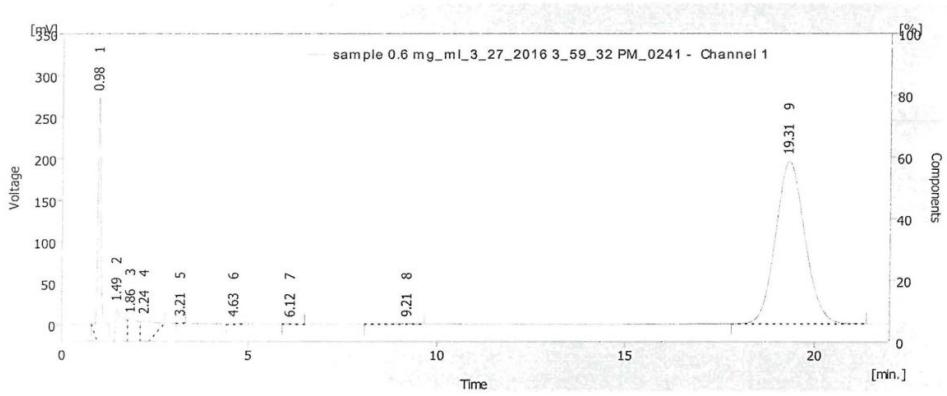


Result Table (Uncal - sample 0.5 mg_ml_3_27_2016 3_36_50 PM_0231 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.977	0.790	2281.926	265.188	15.6		652	2.352	0.000
2	1.490	1.337	1393.929	80.460	9.6		109	1.405	1.420
3	1.870	1.743	1021.302	49.933	7.0		142	1.461	0.635
4	2.237	2.113	717.586	30.303	4.9		62	2.581	0.417
5	4.623	4.433	20.787	1.172	0.1		1087	1.346	2.826
6	6.107	5.890	5.726	0.315	0.0		2457	1.325	2.823
7	9.227	8.907	11.784	0.525	0.1		3639	1.185	5.664
8	19.313	17.893	9138.027	170.101	62.6		3024	1.145	10.030
	Total		14591.067	597.997	100.0				23.814

Sample Info:

Sample ID	: sample 0.6 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

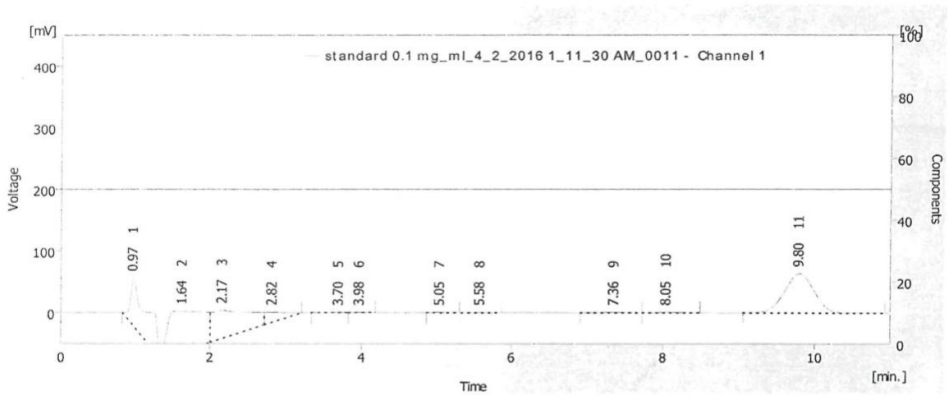


Result Table (Uncal - sample 0.6 mg_ml_3_27_2016 3_59_32 PM_0241 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.977	0.793	2442.932	297.570	15.1		652	2.480	0.000
2	1.487	1.333	1501.061	83.383	9.3		94	1.488	1.337
3	1.863	1.763	957.510	50.391	5.9		157	1.750	0.626
4	2.240	2.113	721.624	30.204	4.5		62	2.526	0.436
5	3.213	3.080	2.440	0.301	0.0		1833	1.000	1.357
6	4.630	4.427	19.504	1.111	0.1		1113	1.184	3.321
7	6.120	5.893	5.838	0.330	0.0		2467	1.278	2.851
8	9.213	8.073	21.279	0.642	0.1		2891	0.701	5.265
9	19.310	17.817	10487.625	195.380	64.9		3023	1.138	9.686
	Total		16159.813	659.310	100.0				24.879

Sample Info:

Sample ID	: standard 0.1 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

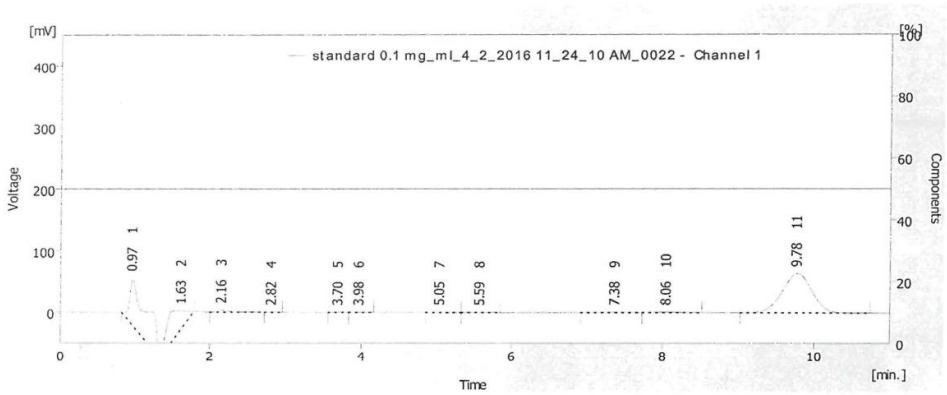


Result Table (Uncal - standard 0.1 mg_ml_4_2_2016 1_11_30 AM_0011 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.973	0.823	1293.807	77.430	18.4		409	1.932	0.000
2	1.637	1.343	2161.066	65.143	30.8		44	1.155	1.129
3	2.170	2.003	1497.931	44.978	21.3		52	2.130	0.488
4	2.823	2.713	295.518	15.767	4.2		184	2.152	0.642
5	3.700	3.330	3.903	0.320	0.1		2963	0.678	1.591
6	3.977	3.827	3.970	0.350	0.1		2427	1.056	0.933
7	5.047	4.863	4.544	0.376	0.1		3648	1.163	3.265
8	5.583	5.327	4.922	0.326	0.1		2917	1.067	1.439
9	7.360	6.913	15.740	0.774	0.2		3191	0.866	3.812
10	8.050	7.727	19.537	0.930	0.3		3106	1.161	1.259
11	9.797	9.060	1716.475	63.563	24.5		2921	0.996	2.688
	Total		7017.413	269.958	100.0				17.247

Sample Info:

Sample ID	: standard 0.1 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

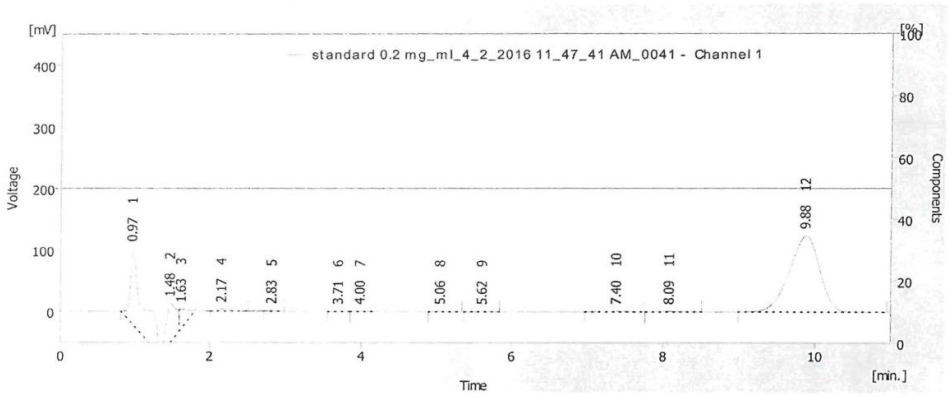


Result Table (Uncal - standard 0.1 mg_ml_4_2_2016 11_24_10 AM_0022 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.970	0.817	1293.662	77.829	33.9		406	1.895	0.000
2	1.630	1.343	706.386	27.812	18.5		125	0.774	1.705
3	2.163	2.000	36.931	3.016	1.0		1013	2.392	1.250
4	2.817	2.713	2.285	0.332	0.1		3422	1.048	2.820
5	3.697	3.553	2.541	0.292	0.1		3863	1.013	4.099
6	3.977	3.830	3.626	0.351	0.1		2916	1.045	1.054
7	5.053	4.860	4.817	0.387	0.1		3422	1.106	3.373
8	5.587	5.330	4.736	0.320	0.1		2920	1.063	1.409
9	7.380	6.920	17.772	0.830	0.5		2886	0.923	3.734
10	8.057	7.730	22.437	1.011	0.6		2827	1.082	1.174
11	9.777	9.027	1721.194	63.832	45.1		2955	0.981	2.602
	Total		3816.388	176.011	100.0				23.222

Sample Info:

Sample ID	: standard 0.2 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

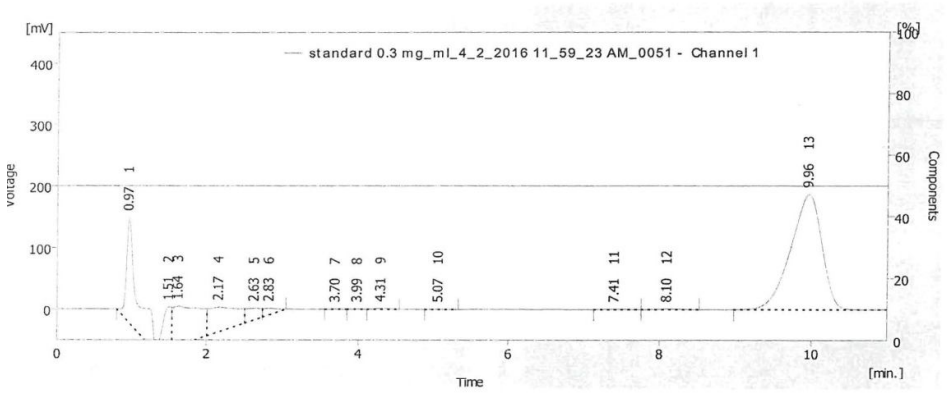


Result Table (Uncal - standard 0.2 mg_ml_4_2_2016 11_47_41 AM_0041 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.970	0.807	1531.380	123.912	26.2		521	1.972	0.000
2	1.483	1.347	588.397	61.841	10.1		406	0.934	2.216
3	1.633	1.593	229.948	29.427	3.9		315	2.625	0.454
4	2.170	2.010	29.873	2.888	0.5		1019	1.459	1.681
5	2.830	2.710	4.410	0.610	0.1		3081	1.081	2.781
6	3.707	3.553	3.030	0.334	0.1		3383	1.024	3.831
7	3.997	3.850	3.643	0.371	0.1		3317	1.011	1.092
8	5.060	4.890	4.641	0.379	0.1		3667	1.267	3.485
9	5.617	5.360	3.334	0.238	0.1		3304	0.955	1.540
10	7.397	6.970	14.963	0.766	0.3		3294	0.892	3.938
11	8.090	7.763	18.032	0.886	0.3		3199	1.119	1.278
12	9.877	8.987	3416.312	123.575	58.4		2878	0.899	2.738
	Total		5847.963	345.225	100.0				25.036

Sample Info:

Sample ID	: standard 0.3 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

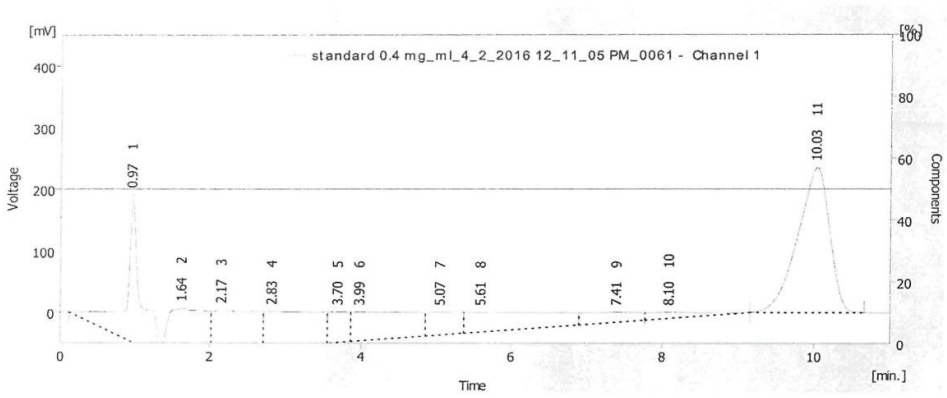


Result Table (Uncal - standard 0.3 mg_ml_4_2_2016 11_59_23 AM_0051 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.970	0.803	1772.063	170.383	16.7		598	2.141	0.000
2	1.507	1.340	532.373	67.231	5.0		744	0.620	2.836
3	1.637	1.543	1526.402	63.108	14.3		69	2.482	0.259
4	2.167	2.007	1020.355	39.599	9.6		105	1.552	0.651
5	2.633	2.503	258.944	18.329	2.4		706	0.897	0.754
6	2.830	2.737	129.314	10.105	1.2		462	1.607	0.427
7	3.703	3.557	2.645	0.317	0.0		3876	1.027	2.290
8	3.993	3.850	2.734	0.308	0.0		4107	0.974	1.194
9	4.307	4.117	17.668	1.541	0.2		2949	1.038	1.109
10	5.073	4.883	4.642	0.379	0.0		3565	1.118	2.340
11	7.407	7.127	12.192	0.695	0.1		3786	1.087	5.697
12	8.103	7.757	19.185	0.926	0.2		3147	1.105	1.319
13	9.960	8.977	5342.241	186.838	50.2		2755	0.836	2.785
	Total		10640.758	559.758	100.0				21.660

Sample Info:

Sample ID	: standard 0.4 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

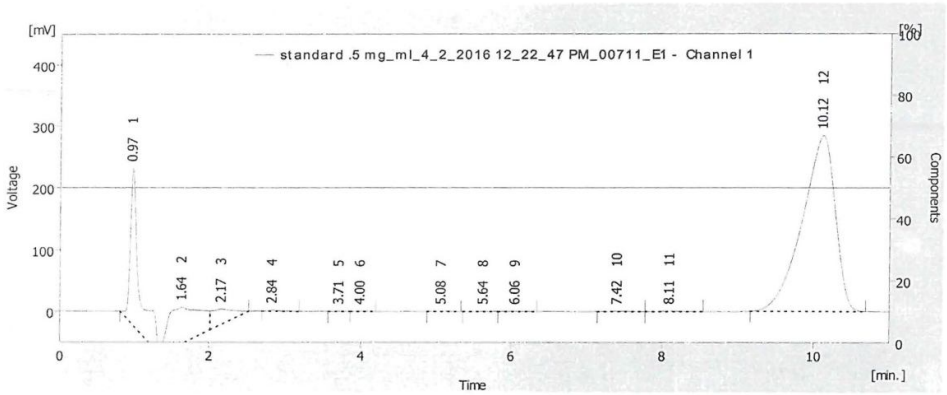


Result Table (Uncal - standard 0.4 mg_ml_4_2_2016 12_11_05 PM_0061 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.973	0.113	3503.124	240.076	13.1		525	0.763	0.000
2	1.640	1.340	2545.331	74.467	9.5		41	1.163	1.124
3	2.167	2.020	2589.247	66.771	9.7		55	2.341	0.483
4	2.830	2.707	2780.102	58.391	10.4		62	3.432	0.510
5	3.703	3.553	916.834	49.511	3.4		791	1.033	0.891
6	3.990	3.863	2571.874	46.848	9.6		89	3.921	0.260
7	5.067	4.857	1122.791	36.889	4.2		533	1.230	0.841
8	5.610	5.373	2461.835	31.817	9.2		75	3.218	0.314
9	7.407	6.897	850.751	15.992	3.2		395	0.859	0.883
10	8.100	7.773	488.232	9.807	1.8		191	1.954	0.363
11	10.033	9.153	6869.837	234.645	25.7		2674	0.798	1.242
	Total		26699.960	865.214	100.0				6.911

Sample Info:

Sample ID	: standard .5 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

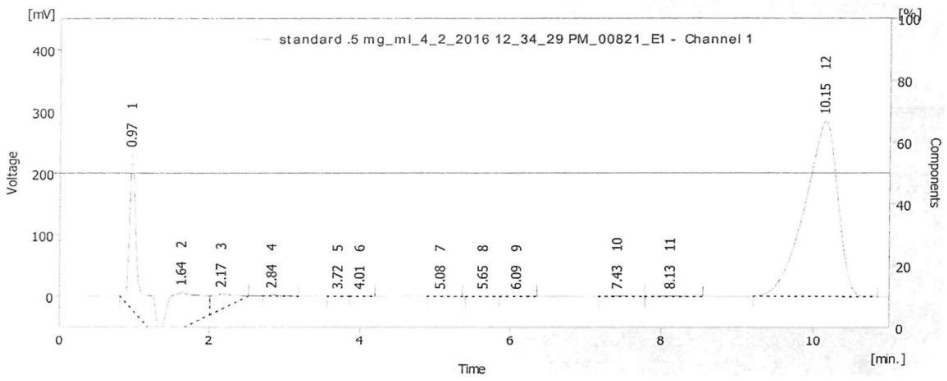


Result Table (Uncal - standard .5 mg_ml_4_2_2016 12_22_47 PM_00711_E1 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.973	0.803	2207.829	253.313	16.8		603	2.407	0.000
2	1.643	1.343	1784.456	58.595	13.6		44	1.138	1.174
3	2.170	2.013	497.865	24.092	3.8		100	1.564	0.570
4	2.837	2.693	14.887	1.697	0.1		2778	1.712	1.236
5	3.713	3.563	3.080	0.342	0.0		3395	1.051	3.739
6	4.000	3.857	3.269	0.330	0.0		3323	1.081	1.080
7	5.080	4.883	4.682	0.382	0.0		3574	1.106	3.508
8	5.640	5.360	4.043	0.264	0.0		2417	0.919	1.406
9	6.063	5.830	5.518	0.343	0.0		3013	1.014	0.943
10	7.417	7.147	11.736	0.672	0.1		3796	1.134	2.939
11	8.107	7.780	17.795	0.862	0.1		3212	1.139	1.313
12	10.117	9.167	8605.146	283.807	65.4		2604	0.766	2.952
	Total		13160.307	624.698	100.0				20.859

Sample Info:

Sample ID	: standard .5 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

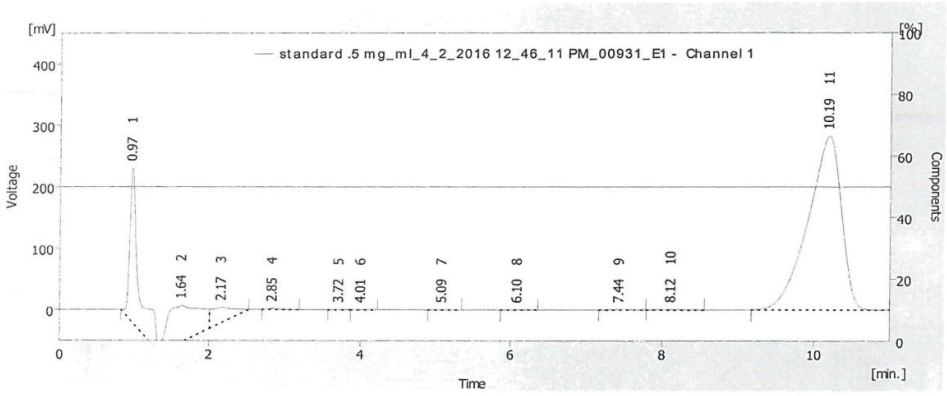


Result Table (Uncal - standard .5 mg_ml_4_2_2016 12_34_29 PM_00821_E1 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.973	0.813	2184.374	252.492	16.6		648	2.481	0.000
2	1.643	1.343	1774.829	58.467	13.5		45	1.132	1.186
3	2.170	2.010	503.816	24.088	3.8		99	1.542	0.570
4	2.840	2.707	15.078	1.712	0.1		2785	1.742	1.235
5	3.717	3.567	3.047	0.339	0.0		3558	1.064	3.785
6	4.007	3.863	3.101	0.315	0.0		3623	1.070	1.128
7	5.080	4.893	4.596	0.371	0.0		3574	1.173	3.551
8	5.647	5.407	4.168	0.261	0.0		2053	0.992	1.355
9	6.090	5.857	5.303	0.333	0.0		3202	0.986	0.957
10	7.430	7.170	11.387	0.658	0.1		3901	1.145	2.965
11	8.130	7.787	17.906	0.860	0.1		3168	1.092	1.332
12	10.153	9.210	8628.041	283.548	65.6		2585	0.766	2.948
	Total		13155.647	623.445	100.0				21.012

Sample Info:

Sample ID	: standard .5 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

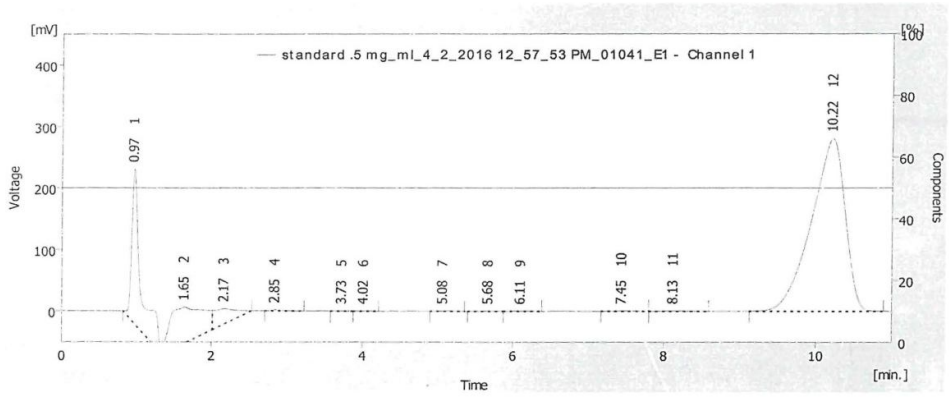


Result Table (Uncal - standard .5 mg_ml_4_2_2016 12_46_11 PM_00931_E1 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.973	0.823	2159.775	250.959	16.4		648	2.560	0.000
2	1.643	1.343	1769.469	58.404	13.4		46	1.140	1.192
3	2.173	2.010	513.776	24.204	3.9		97	1.531	0.572
4	2.847	2.703	15.345	1.717	0.1		2656	1.706	1.222
5	3.723	3.573	3.121	0.344	0.0		3413	1.064	3.695
6	4.013	3.870	3.148	0.315	0.0		3345	1.081	1.092
7	5.090	4.897	4.591	0.370	0.0		3472	1.137	3.465
8	6.097	5.867	4.179	0.295	0.0		3782	1.092	2.720
9	7.443	7.170	11.520	0.658	0.1		3735	1.110	3.056
10	8.123	7.793	18.046	0.865	0.1		3162	1.151	1.280
11	10.193	9.170	8653.370	282.352	65.8		2569	0.762	3.003
	Total		13156.341	620.483	100.0				21.298

Sample Info:

Sample ID	: standard .5 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

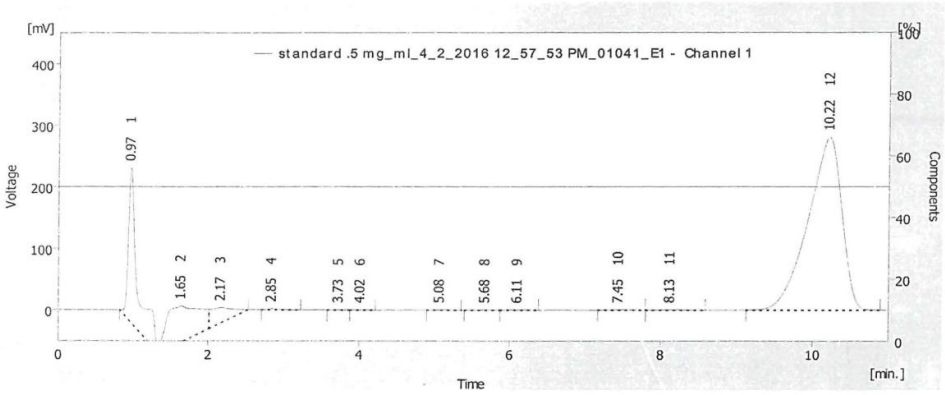


Result Table (Uncal - standard .5 mg_ml_4_2_2016 12_57_53 PM_01041_E1 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.973	0.823	2159.260	250.933	16.4		648	2.560	0.000
2	1.647	1.343	1773.425	58.182	13.5		45	1.132	1.192
3	2.173	2.013	516.517	24.411	3.9		96	1.573	0.565
4	2.847	2.707	15.547	1.715	0.1		2656	1.773	1.216
5	3.730	3.577	3.062	0.337	0.0		3426	1.064	3.723
6	4.020	3.877	3.103	0.309	0.0		3223	1.070	1.081
7	5.080	4.907	4.615	0.369	0.0		3574	1.245	3.411
8	5.680	5.403	4.280	0.263	0.0		1821	0.914	1.379
9	6.107	5.873	5.521	0.336	0.0		2979	1.000	0.873
10	7.450	7.173	11.707	0.665	0.1		3742	1.088	2.882
11	8.130	7.803	18.372	0.871	0.1		3106	1.157	1.274
12	10.217	9.127	8659.892	279.999	65.7		2510	0.762	2.991
	Total		13175.299	618.390	100.0				20.586

Sample Info:

Sample ID	: standard .5 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

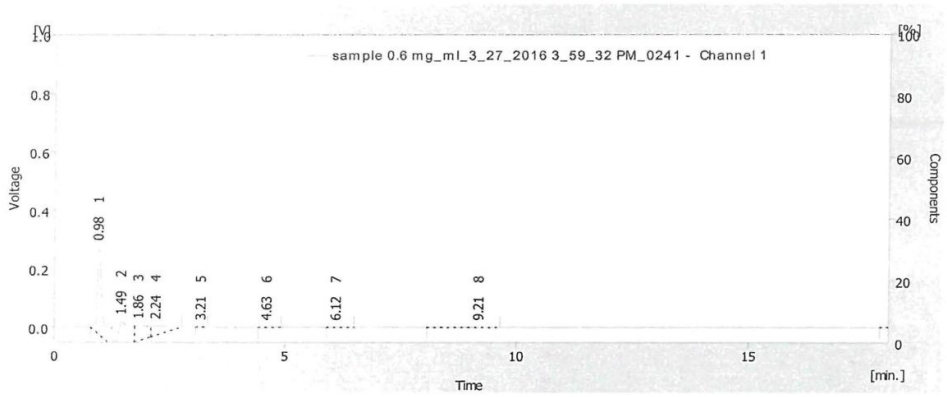


Result Table (Uncal - standard .5 mg_ml_4_2_2016 12_57_53 PM_01041_E1 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.973	0.823	2159.260	250.933	16.4		648	2.560	0.000
2	1.647	1.343	1773.425	58.182	13.5		45	1.132	1.192
3	2.173	2.013	516.517	24.411	3.9		96	1.573	0.565
4	2.847	2.707	15.547	1.715	0.1		2656	1.773	1.216
5	3.730	3.577	3.062	0.337	0.0		3426	1.064	3.723
6	4.020	3.877	3.103	0.309	0.0		3223	1.070	1.081
7	5.080	4.907	4.615	0.369	0.0		3574	1.245	3.411
8	5.680	5.403	4.280	0.263	0.0		1821	0.914	1.379
9	6.107	5.873	5.521	0.336	0.0		2979	1.000	0.873
10	7.450	7.173	11.707	0.665	0.1		3742	1.088	2.882
11	8.130	7.803	18.372	0.871	0.1		3106	1.157	1.274
12	10.217	9.127	8659.892	279.999	65.7		2510	0.762	2.991
	Total		13175.299	618.390	100.0				20.586

Sample Info:

Sample ID	: sample 0.6 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

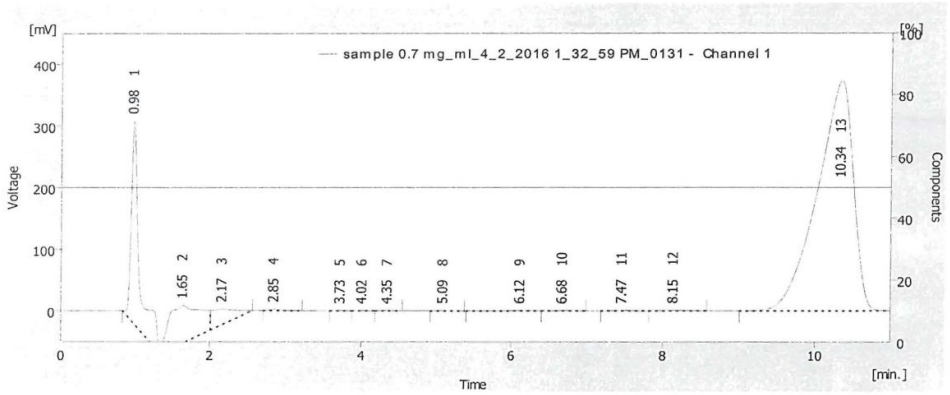


Result Table (Uncal - sample 0.6 mg_ml_3_27_2016 3_59_32 PM_0241 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.977	0.793	2442.932	297.570	15.1		652	2.480	0.000
2	1.487	1.333	1501.061	83.383	9.3		94	1.488	1.337
3	1.863	1.763	957.510	50.391	5.9		157	1.750	0.626
4	2.240	2.113	721.624	30.204	4.5		62	2.526	0.436
5	3.213	3.080	2.440	0.301	0.0		1833	1.000	1.357
6	4.630	4.427	19.504	1.111	0.1		1113	1.184	3.321
7	6.120	5.893	5.838	0.330	0.0		2467	1.278	2.851
8	9.213	8.073	21.279	0.642	0.1		2891	0.701	5.265
9	19.310	17.817	10487.625	195.380	64.9		3023	1.138	9.686
	Total		16159.813	659.310	100.0				24.879

Sample Info:

Sample ID	: sample 0.7 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

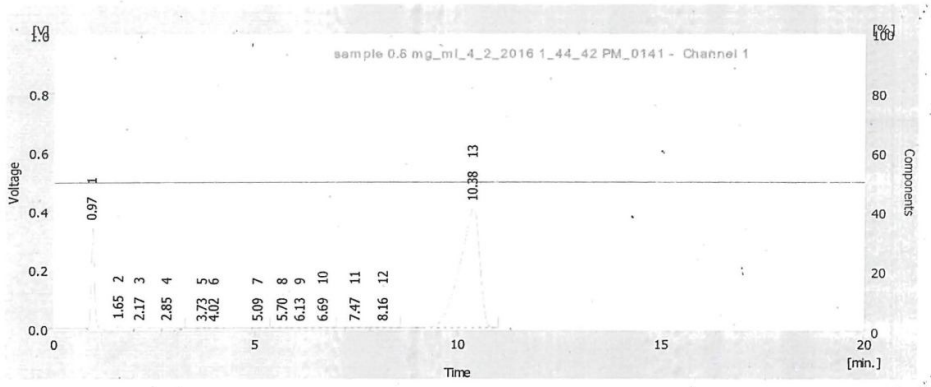


Result Table (Uncal - sample 0.7 mg_ml_4_2_2016 1_32_59 PM_0131 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.977	0.827	2578.312	328.409	14.9		652	2.520	0.000
2	1.647	1.343	1788.467	60.595	10.3		46	1.126	1.192
3	2.170	2.010	551.682	24.795	3.2		85	1.667	0.548
4	2.850	2.703	20.718	2.292	0.1		2663	1.721	1.174
5	3.727	3.583	2.866	0.321	0.0		3577	1.095	3.739
6	4.017	3.873	2.820	0.302	0.0		3802	0.988	1.141
7	4.350	4.180	3.374	0.312	0.0		3119	1.067	1.168
8	5.093	4.917	4.375	0.357	0.0		3593	1.234	2.288
9	6.123	5.397	14.787	0.558	0.1		2528	0.713	2.497
10	6.683	6.403	6.101	0.306	0.0		2272	0.964	1.072
11	7.467	7.183	11.044	0.628	0.1		3758	1.066	1.499
12	8.147	7.817	17.756	0.848	0.1		3119	1.131	1.274
13	10.343	9.007	12357.117	372.177	71.2		2249	0.721	3.026
	Total		17359.418	791.899	100.0				20.618

Sample Info:

Sample ID	: sample 0.8 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

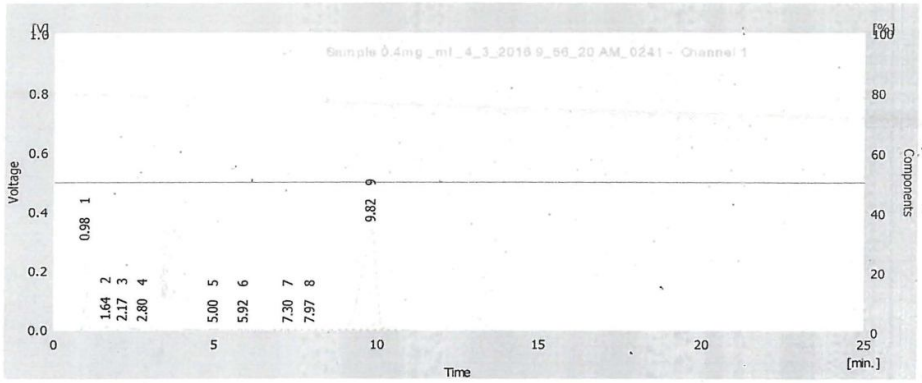


Result Table (Uncal - sample 0.8 mg_ml_4_2_2016 1_44_42 PM_0141 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	W05 [min]	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.973	0.827	2790.830	365.160	13.5	0.09	603	2.604	0.000
2	1.647	1.340	2090.902	67.457	10.1	0.58	45	1.119	1.180
3	2.173	2.010	1440.480	43.421	7.0	0.69	54	2.122	0.488
4	2.850	2.703	398.220	18.789	1.9	0.58	132	1.909	0.625
5	3.727	3.573	3.287	0.347	0.0	0.15	3272	1.063	1.404
6	4.017	3.877	3.349	0.318	0.0	0.17	2975	1.143	1.048
7	5.093	4.920	4.354	0.354	0.0	0.20	3716	1.208	3.434
8	5.697	5.417	6.235	0.363	0.0	0.32	1720	0.892	1.369
9	6.127	5.890	10.787	0.599	0.1	0.29	2530	1.085	0.832
10	6.687	6.403	6.902	0.349	0.0	0.33	2321	0.982	1.077
11	7.470	7.197	10.812	0.618	0.1	0.29	3762	1.111	1.507
12	8.163	7.817	17.012	0.818	0.1	0.34	3194	1.062	1.306
13	10.383	8.977	13942.339	407.841	67.3	0.53	2153	0.707	3.023
	Total		20725.509	906.434	100.0				17.293

Sample Info:

Sample ID	: Sample 0.4mg /ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

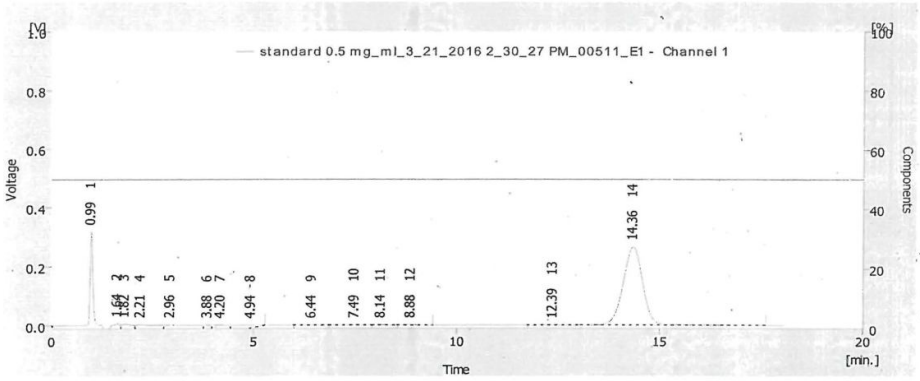


Result Table (Uncal - Sample 0.4mg _ml_4_3_2016 9_56_20 AM_0241 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	W05 [min]	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.977	0.833	2470.182	299.451	14.2	0.09	652	2.540	0.000
2	1.643	1.343	2352.543	74.585	13.5	0.58	45	1.128	1.180
3	2.167	2.003	1682.107	50.518	9.6	0.68	57	2.071	0.493
4	2.803	2.680	580.397	24.656	3.3	0.69	91	2.676	0.550
5	5.000	4.857	4.065	0.348	0.0	0.18	4438	1.359	2.991
6	5.920	5.743	4.393	0.331	0.0	0.22	3893	1.184	2.714
7	7.300	6.837	19.276	0.827	0.1	0.33	2767	0.954	2.961
8	7.973	7.693	22.189	0.956	0.1	0.35	2875	1.357	1.174
9	9.823	8.620	10306.803	343.640	59.1	0.47	2420	0.733	2.662
	Total		17441.954	795.314	100.0				14.724

Sample Info:

Sample ID	: standard 0.5 mg/ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		

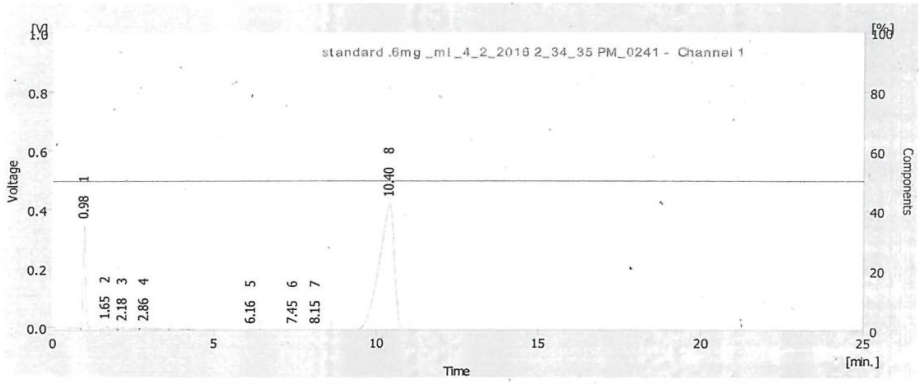


Result Table (Uncal - standard 0.5 mg_ml_3_21_2016 2_30_27 PM_00511_E1 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	W05 [min]	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.987	0.007	5824.120	401.880	20.8	0.09	666	0.722	0.000
2	1.643	1.347	2090.772	112.634	7.5	0.32	149	0.634	1.905
3	1.817	1.720	2164.224	105.159	7.7	0.36	144	1.845	0.304
4	2.213	2.077	3616.613	92.372	12.9	0.72	53	2.622	0.436
5	2.957	2.793	3463.265	70.222	12.3	0.99	50	3.020	0.515
6	3.883	3.780	507.580	41.369	1.8	0.21	1956	1.000	0.916
7	4.203	3.987	868.241	32.321	3.1	0.46	463	1.062	0.566
8	4.943	4.447	616.541	10.157	2.2	0.84	192	0.829	0.672
9	6.437	5.990	11.092	0.602	0.0	0.27	3149	0.916	1.588
10	7.493	6.863	16.618	0.513	0.1	0.61	836	0.885	1.417
11	8.143	7.917	24.689	1.222	0.1	0.28	4576	1.382	0.859
12	8.877	8.543	33.385	1.541	0.1	0.33	4091	1.100	1.419
13	12.393	11.760	16.657	0.333	0.1	0.89	1082	1.076	3.420
14	14.360	13.073	8807.654	264.478	31.4	0.51	4392	0.971	1.662
	Total		28061.450	1134.803	100.0				15.678

Sample Info:

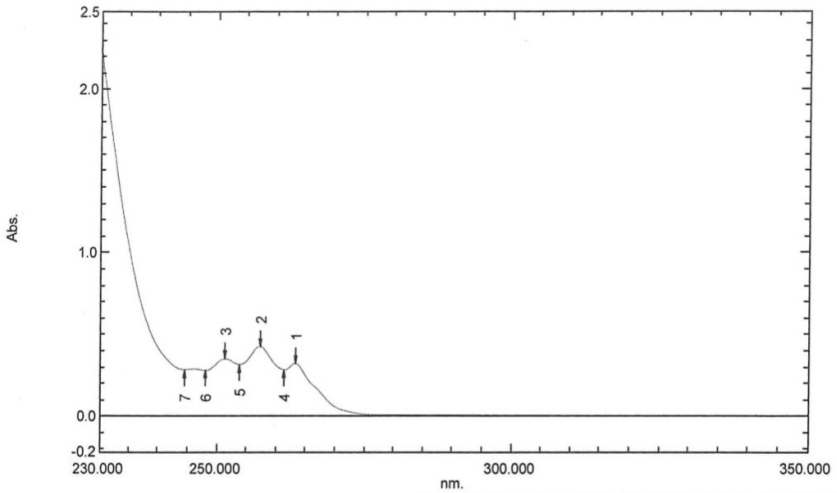
Sample ID	: standard .6mg /ml	Amount	: 0
Sample	: hyoscine butyl bromide	ISTD Amount	: 0
Inj. Volume [mL]	: 0.02	Dilution	: 1
Column	: C18 (15cm *.3.9 mm) 10 Mm	Detection	: 210
Mobile Phase	: HCl:MeOH:Sodium dodecyl sulfate	Temperature	: 28
Flow Rate	: 2	Pressure	:
Note	:		



Result Table (Uncal - standard .6mg _ml_4_2_2016 2_34_35 PM_0241 - Channel 1)

	Reten. Time [min]	Start Time [min]	Area [mV.s]	Height [mV]	Area [%]	W05 [min]	Efficiency [th.pl]	Symmetry/Tailing [-]	resolution
1	0.980	0.860	2882.235	369.177	12.8	0.09	657	2.520	0.000
2	1.653	1.347	2551.245	80.664	11.3	0.59	44	1.132	1.168
3	2.183	2.020	1973.894	56.407	8.7	0.69	56	2.102	0.490
4	2.857	2.707	1067.736	33.013	4.7	0.98	47	3.111	0.477
5	6.157	5.973	6.820	0.469	0.0	0.24	3546	1.235	3.183
6	7.450	7.233	9.818	0.589	0.0	0.26	4434	1.284	3.012
7	8.150	7.843	17.136	0.823	0.1	0.33	3312	1.196	1.384
8	10.397	9.080	14082.776	431.294	62.3	0.51	2333	0.711	3.156
	Total		22591.661	972.436	100.0				12.871

Data Set: 09-09-2015 Hyosin butylbromide stock.spc - RawData



Measurement Properties

Wavelength Range (nm.):	230.000 to 350.000
Scan Speed:	Fast
Sampling Interval:	0.1
Auto Sampling Interval:	Disabled
Scan Mode:	Auto

Instrument Properties

Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	340.0 nm
S/R Exchange:	Normal

Attachment Properties

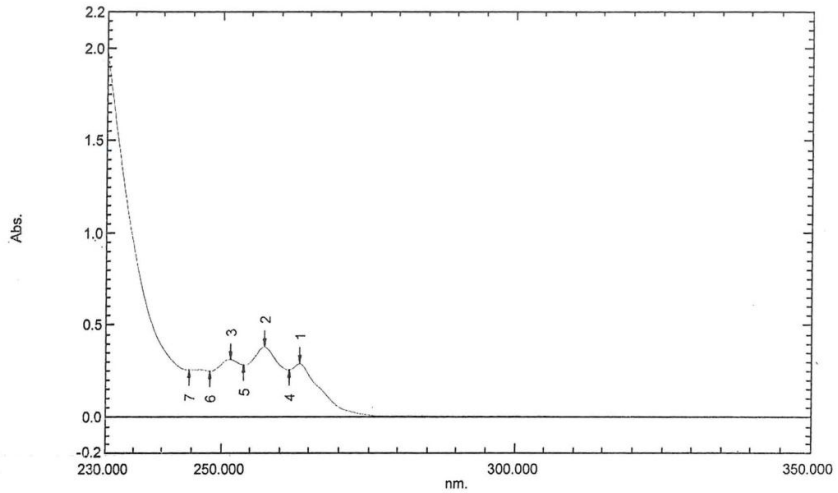
Attachment:	None

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

No.	P/V	Wavelength	Abs.	Description
1	P	263.189	0.3	
2	P	257.159	0.4	
3	P	251.301	0.3	
4	V	261.303	0.3	
5	V	253.784	0.3	
6	V	247.927	0.3	
7	V	244.386	0.3	

Data Set: 09-09-2015 Hyosin butylbromide pH1.spc - RawData



Measurement Properties
Wavelength Range (nm.): 230.000 to 350.000
Scan Speed: Fast
Sampling Interval: 0.1
Auto Sampling Interval: Disabled
Scan Mode: Auto

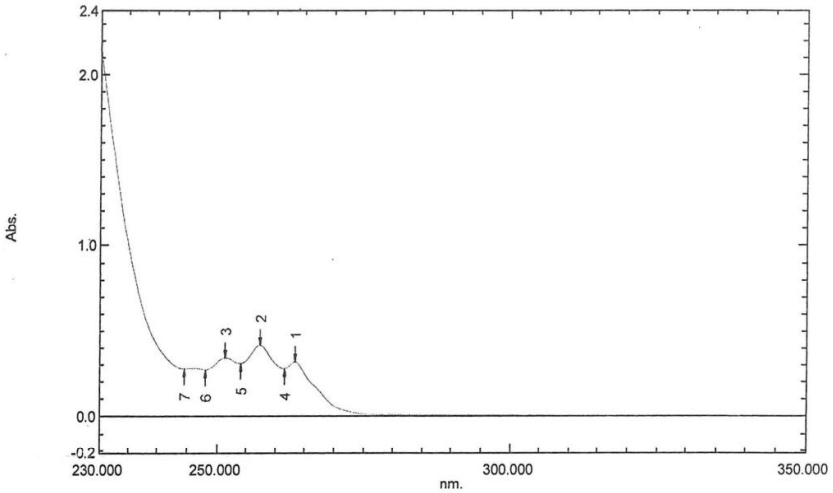
Instrument Properties
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

No.	P/V	Wavelength	Abs.	Description
1	●	263.300	0.3	
2	●	257.200	0.4	
3	●	251.400	0.3	
4	⊕	261.400	0.3	
5	⊕	253.800	0.3	
6	⊕	248.000	0.2	
7	⊕	244.400	0.2	

Data Set: 09-09-2015 Hyosin butylbromide pH2.spc - RawData



Measurement Properties

Wavelength Range (nm.):	230.000 to 350.000
Scan Speed:	Fast
Sampling Interval:	0.1
Auto Sampling Interval:	Disabled
Scan Mode:	Auto

Instrument Properties

Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	340.0 nm
S/R Exchange:	Normal

Attachment Properties

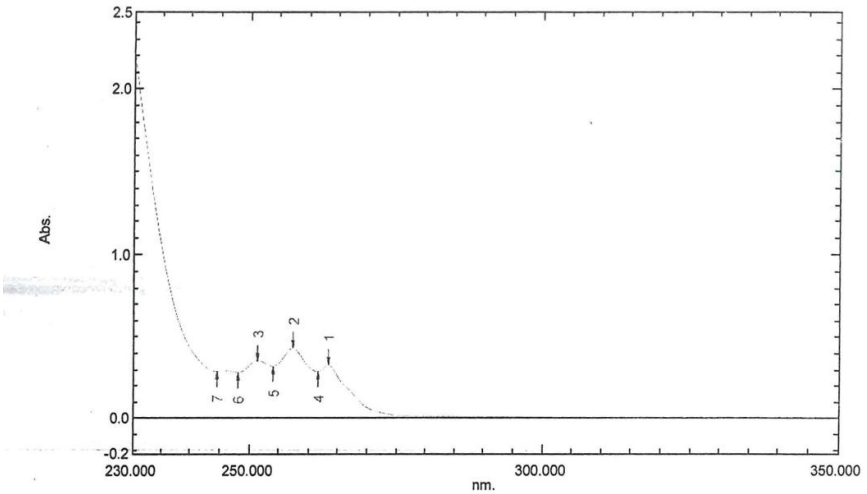
Attachment:	None

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

No.	P/V	Wavelength	Abs.	Description
1	●	263.246	0.3	
2	●	257.179	0.4	
3	●	251.339	0.3	
4	▼	261.335	0.3	
5	▼	253.844	0.3	
6	▼	247.990	0.3	
7	▼	244.420	0.3	

Data Set: 09-09-2015 Hyosin butylbromide pH3.spc - RawData



Measurement Properties

Wavelength Range (nm.):	230.000 to 350.000
Scan Speed:	Fast
Sampling Interval:	0.1
Auto Sampling Interval:	Disabled
Scan Mode:	Auto

Instrument Properties

Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	340.0 nm
S/R Exchange:	Normal

Attachment Properties

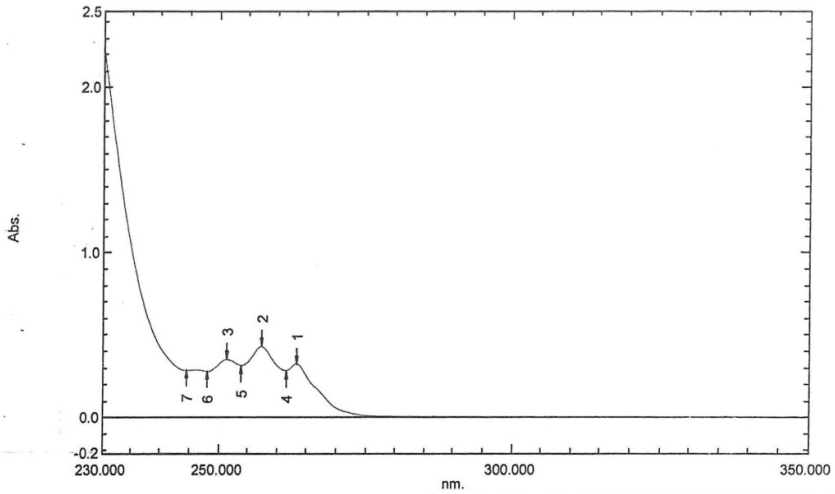
Attachment:	None

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

No.	P/V	Wavelength	Abs.	Description
1	●	263.252	0.3	
2	●	257.204	0.4	
3	●	251.314	0.4	
4	✚	261.341	0.3	
5	✚	253.847	0.3	
6	✚	247.984	0.3	
7	✚	244.424	0.3	

Data Set: 09-09-2015 Hyosin butylbromide pH4.spc - RawData



Measurement Properties

Wavelength Range (nm.):	230.000 to 350.000
Scan Speed:	Fast
Sampling Interval:	0.1
Auto Sampling Interval:	Disabled
Scan Mode:	Auto

Instrument Properties

Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	340.0 nm
S/R Exchange:	Normal

Attachment Properties

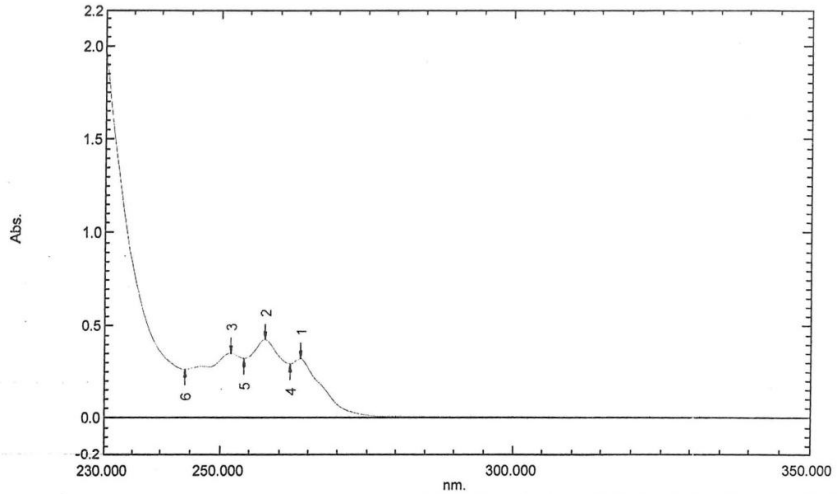
Attachment:	None

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

No.	P/V	Wavelength	Abs.	Description
1	●	263.204	0.3	
2	●	257.184	0.4	
3	●	251.301	0.3	
4	◆	261.341	0.3	
5	◆	253.769	0.3	
6	◆	247.960	0.3	
7	◆	244.395	0.3	

Data Set: 09-09-2015 Hyosin butylbromide pH5.spc - RawData



Measurement Properties

Wavelength Range (nm.):	230.000 to 350.000
Scan Speed:	Fast
Sampling Interval:	0.1
Auto Sampling Interval:	Disabled
Scan Mode:	Auto

Instrument Properties

Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	340.0 nm
S/R Exchange:	Normal

Attachment Properties

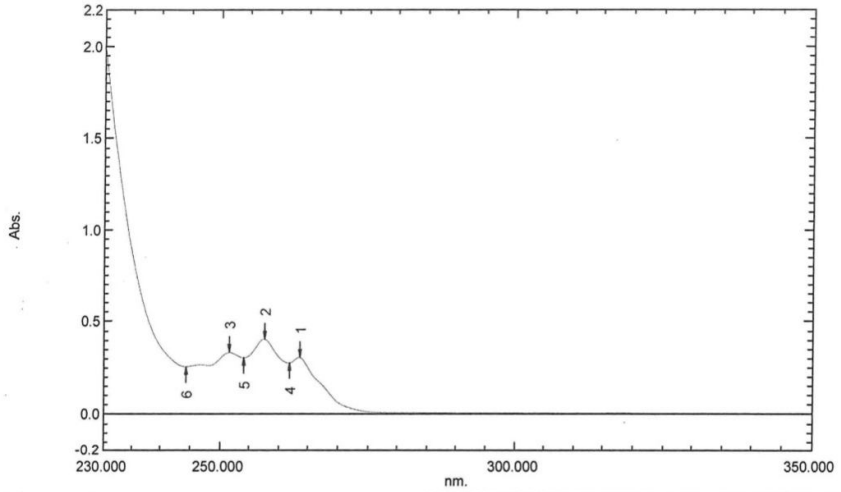
Attachment:	None

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

No.	P/V	Wavelength	Abs.	Description
1	●	263.365	0.3	
2	●	257.415	0.4	
3	●	251.652	0.3	
4	▼	261.700	0.3	
5	▼	254.014	0.3	
6	▼	243.876	0.3	

Data Set: 09-09-2015 Hyosin butylbromide pH6.spc - RawData



Measurement Properties
Wavelength Range (nm.): 230.000 to 350.000
Scan Speed: Fast
Sampling Interval: 0.1
Auto Sampling Interval: Disabled
Scan Mode: Auto

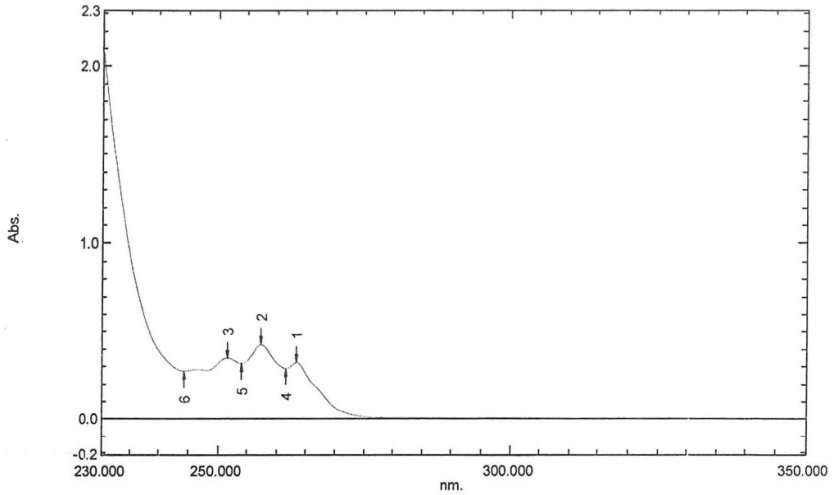
Instrument Properties
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

No.	P/V	Wavelength	Abs.	Description
1		263.331	0.3	
2		257.352	0.4	
3		251.502	0.3	
4		261.550	0.3	
5		253.969	0.3	
6		244.108	0.3	

Data Set: 09-09-2015 Hyosin butylbromide pH7.spc - RawData



Measurement Properties
Wavelength Range (nm.): 230.000 to 350.000
Scan Speed: Fast
Sampling Interval: 0.1
Auto Sampling Interval: Disabled
Scan Mode: Auto

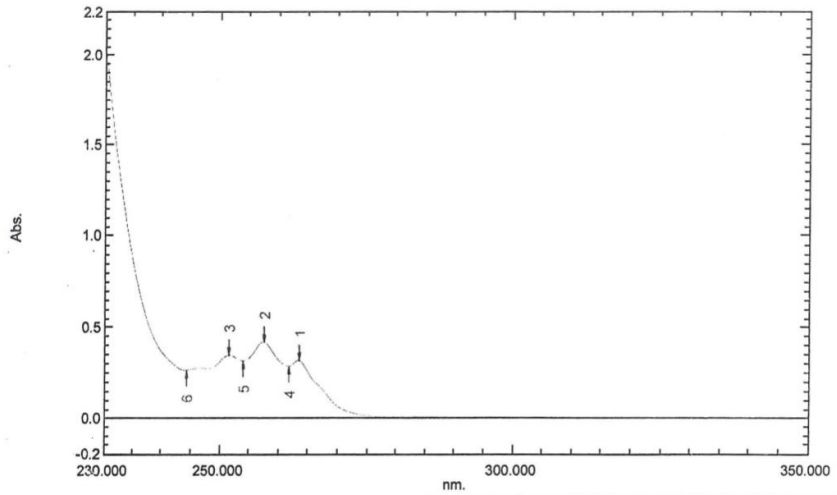
Instrument Properties
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

No.	P/V	Wavelength	Abs.	Description
1	●	263.296	0.3	
2	●	257.289	0.4	
3	●	251.448	0.3	
4	⊖	261.484	0.3	
5	⊖	253.915	0.3	
6	⊖	244.144	0.3	

Data Set: 09-09-2015 Hyosin butylbromide pH8.spc - RawData



Measurement Properties
Wavelength Range (nm.): 230.000 to 350.000
Scan Speed: Fast
Sampling Interval: 0.1
Auto Sampling Interval: Disabled
Scan Mode: Auto

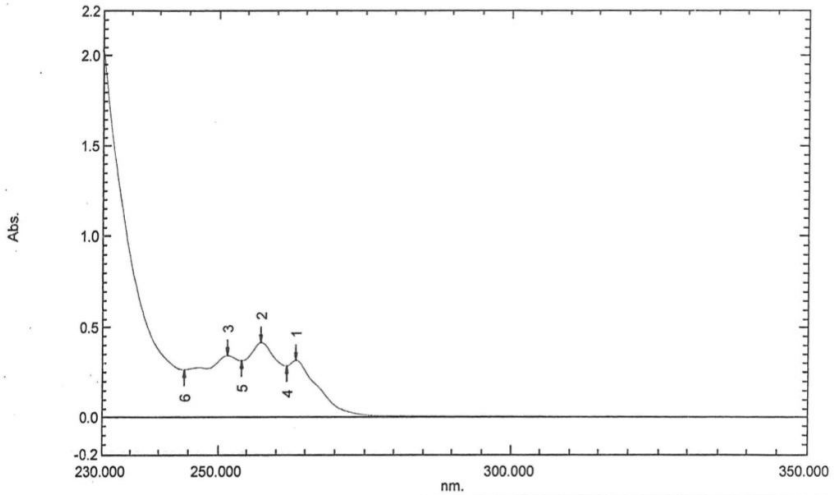
Instrument Properties
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

No.	P/V	Wavelength	Abs.	Description
1	●	263.323	0.3	
2	●	257.349	0.4	
3	●	251.467	0.3	
4	◆	261.564	0.3	
5	◆	253.956	0.3	
6	◆	244.087	0.3	

Data Set: 09-09-2015 Hyosin butylbromide pH9.spc - RawData



Measurement Properties

Wavelength Range (nm.):	230.000 to 350.000
Scan Speed:	Fast
Sampling Interval:	0.1
Auto Sampling Interval:	Disabled
Scan Mode:	Auto

Instrument Properties

Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	340.0 nm
S/R Exchange:	Normal

Attachment Properties

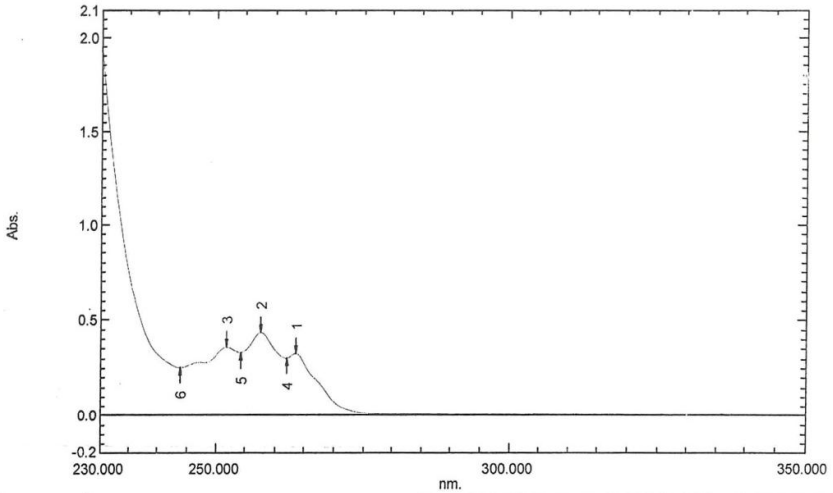
Attachment:	None

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

No.	P/V	Wavelength	Abs.	Description
1	●	263.316	0.3	
2	●	257.342	0.4	
3	●	251.503	0.3	
4	▼	261.553	0.3	
5	▼	253.947	0.3	
6	▼	244.102	0.3	

Data Set: 09-09-2015 Hyosin butylbromide pH10.spc - RawData



Measurement Properties

Wavelength Range (nm.):	230.000 to 350.000
Scan Speed:	Fast
Sampling Interval:	0.1
Auto Sampling Interval:	Disabled
Scan Mode:	Auto

Instrument Properties

Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	340.0 nm
S/R Exchange:	Normal

Attachment Properties

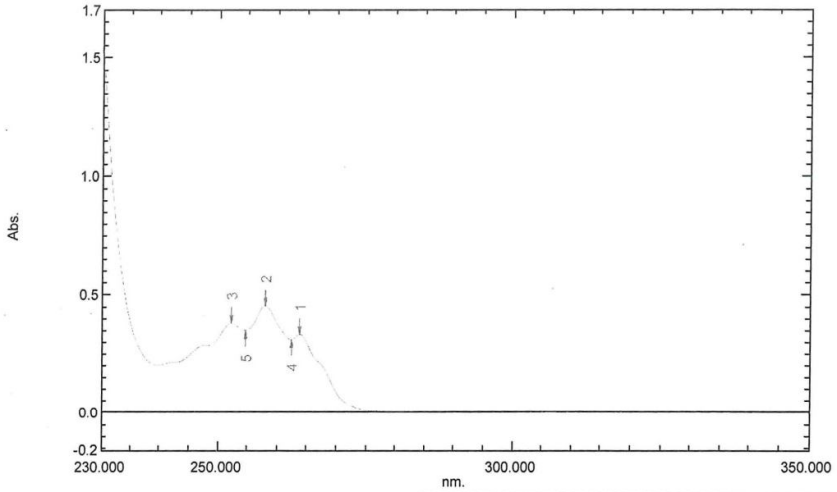
Attachment:	None

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

No.	P/V	Wavelength	Abs.	Description
1	●	263.416	0.3	
2	●	257.483	0.4	
3	●	251.718	0.4	
4	⊕	261.800	0.3	
5	⊕	254.081	0.3	
6	⊕	243.621	0.3	

Data Set: 09-09-2015 Hyosin butylbromide pH 11.spc - RawData



No.	P/V	Wavelength	Abs.	Description
1		263.702	0.3	
2		257.848	0.5	
3		252.169	0.4	
4		262.248	0.3	
5		254.532	0.3	

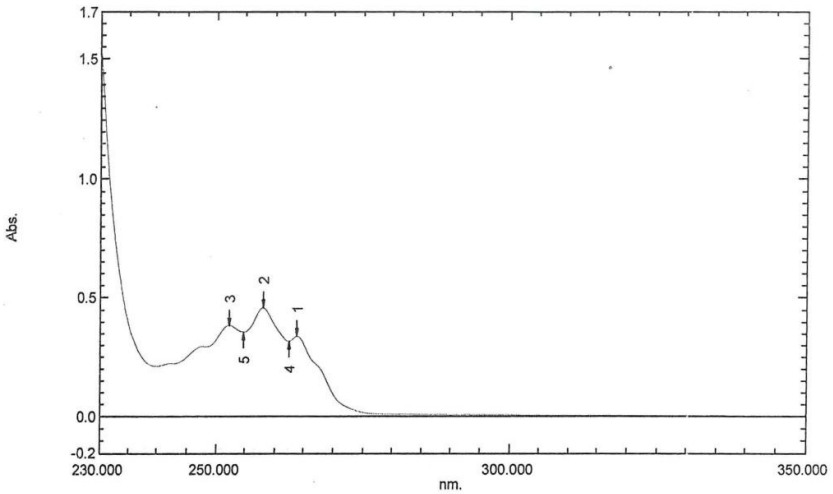
Measurement Properties
Wavelength Range (nm.): 230.000 to 350.000
Scan Speed: Fast
Sampling Interval: 0.1
Auto Sampling Interval: Disabled
Scan Mode: Auto

Instrument Properties
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

Data Set: 09-09-2015 Hyosin butylbromide pH 12.spc - RawData



No.	P/V	Wavelength	Abs.	Description
1	●	263.713	0.3	
2	●	257.837	0.5	
3	●	252.168	0.4	
4	⊖	262.232	0.3	
5	⊖	254.585	0.4	

Measurement Properties

Wavelength Range (nm.):	230.000 to 350.000
Scan Speed:	Fast
Sampling Interval:	0.1
Auto Sampling Interval:	Disabled
Scan Mode:	Auto

Instrument Properties

Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	340.0 nm
S/R Exchange:	Normal

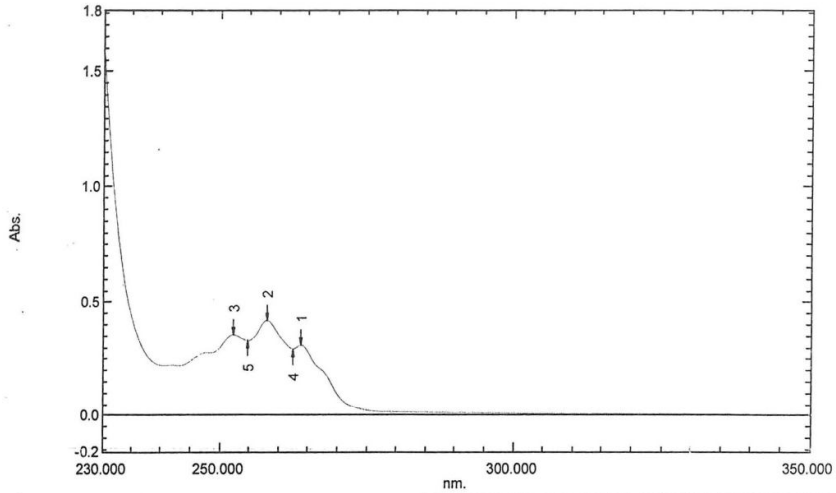
Attachment Properties

Attachment:	None

Sample Preparation Properties

Weight:
Volume:
Dilution:
Path Length:
Additional Information:

Data Set: 09-09-2015 Hyosin butylbromide pH13.spc - RawData



Measurement Properties

Wavelength Range (nm.):	230.000 to 350.000
Scan Speed:	Fast
Sampling Interval:	0.1
Auto Sampling Interval:	Disabled
Scan Mode:	Auto

Instrument Properties

Instrument Type:	UV-1800 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	340.0 nm
S/R Exchange:	Normal

Attachment Properties

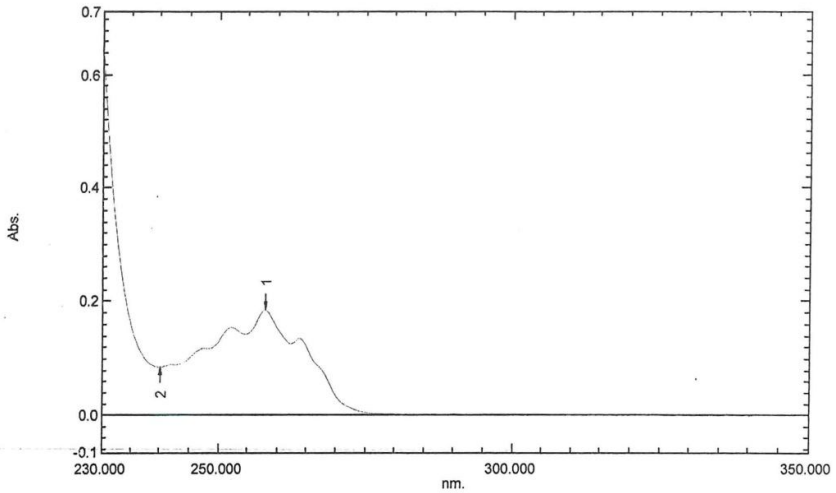
Attachment:	None

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

No.	P/V	Wavelength	Abs.	Description
1	●	263.703	0.3	
2	●	257.856	0.4	
3	●	252.207	0.4	
4	◆	262.298	0.3	
5	◆	254.534	0.3	

Data Set: 09-09-2015 Hyosin butylbromide pH14.spc - RawData



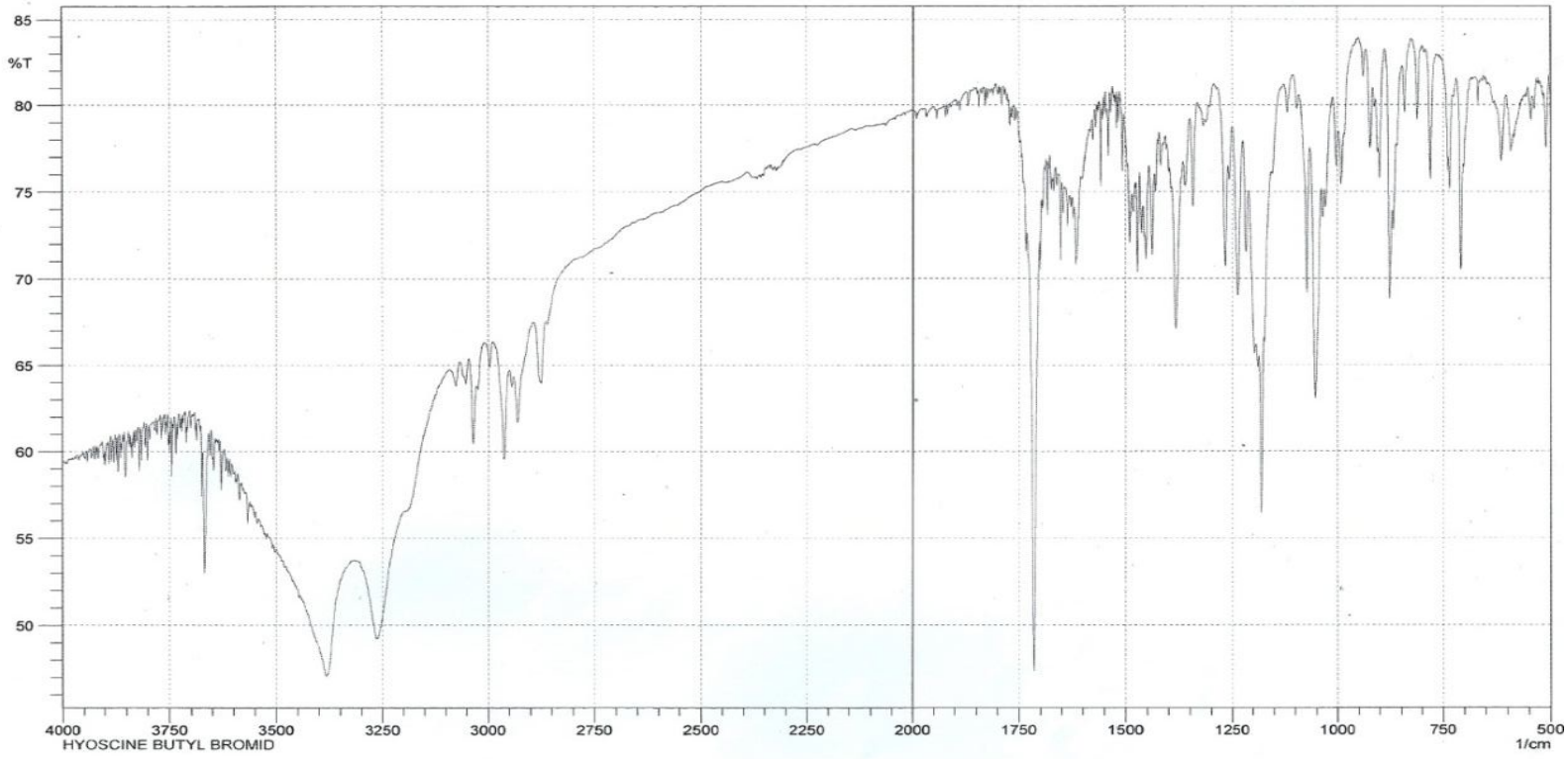
No.	P/V	Wavelength	Abs.	Description
1		257.796	0.2	
2		239.991	0.1	

Measurement Properties
Wavelength Range (nm.): 230.000 to 350.000
Scan Speed: Fast
Sampling Interval: 0.1
Auto Sampling Interval: Disabled
Scan Mode: Auto

Instrument Properties
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:


%T
85
80
75
70
65
60
55
50
4000
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3500
3250
3000
2750
2500
2250
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1750
1500
1250
1000
750
500
1/cm
HYOSCINE BUTYL BROMID